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FOLIAR PENETRATION OF 2,4-D AND PICLORAM IN
POPULUS TREMULOIDES MICHX., P. BALSAMIFERA L,
AND CIRSIIUM ARVENSE (L.) SCOP.

BY

MAHENDRA P. SHARMA



A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF PLANT SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Foliar Penetration of 2,4-D and Picloram in Populus tremuloides Michx., P. balsamifera L. and Cirsium arvense (L.) Scop.", submitted by Mahendra P. Sharma in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The quantitative nature of foliar penetration of 4-amino-3,5,6-trichloropicolinic acid (picloram), and amine and ester formulations of 2,4-dichlorophenoxyacetic acid (2,4-D) in detached leaves of aspen poplar (Populus tremuloides Michx.) and balsam poplar (Populus balsamifera L.) and intact plants of Canada thistle (Cirsium arvense (L.) scop.) was studied by spectrophotometric and radioassay methods. The influence of a number of factors on penetration was examined. These factors included added surfactant, relative humidity, stage of plant growth, adaxial or abaxial leaf surface, temperature, removal of cuticular waxes, and concentration of herbicide solution.

In general, added surfactant (Atlox 210) at 1% (v/v), and high relative humidity enhanced the penetration of both picloram and 2,4-D in all experiments with aspen poplar and Canada thistle. The influence of added surfactant was greater for picloram and 2,4-D amine than for 2,4-D ester.

Penetration of picloram and 2,4-D amine from the adaxial surface of aspen poplar leaves occurred readily in young leaves in early June. There was an increase in penetration in early July and then a decrease in August and September to a level equal to or less than that in June. Penetration from the abaxial surface of aspen poplar leaves was nearly equal in June and July, but there was a gradual decrease in August and September.

Effects of added surfactant and relative humidity were similar at the four growth stages examined. At each growth stage, penetration of picloram and 2,4-D amine was greater from the abaxial than from the adaxial surface of aspen poplar leaves. Ester formulation of 2,4-D

penetrated equally readily from both surfaces.

An increase in temperature from 10° to 25.5° or to 40.5° C resulted in sharply increased penetration of both picloram and 2,4-D under both low and high humidity in all three species tested. Movement of picloram within the leaf also was much more extensive at the higher temperatures.

Partial removal of cuticular waxes from the adaxial surface of aspen poplar leaves with chloroform resulted in a sharp increase in penetration of picloram and 2,4-D.

Variations in the concentration of herbicide in treatment droplets did not influence the extent of penetration.

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INTRODUCTION

Various formulations of the systemic herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) have been used for many years with varying success to control a number of woody plants and herbaceous perennial weeds (15, 22, 26). In recent years picloram (4-amino-3,5,6-trichloropicolinic acid) has shown considerable promise for the control of such 2,4-D resistant species (1, 15, 28, 30, 34, 36, 67, 68).

Differences in susceptibility to picloram between some of these species, for example, aspen poplar (Populus tremuloides Michx.) and balsam poplar (Populus balsamifera L.) (15) have not yet been fully explained. Furthermore, reports of marked increases in effectiveness following the addition of a specific surfactant (15, 29) led to the suggestion that penetration into leaves of these woody species may be limiting the herbicide's effectiveness. Additional information could provide added support for existing recommendation regarding the best time of applying spray treatments in a brush control program. Because penetration of herbicides from the surface of the leaf to the interior is the first step in the chain of events leading to a response by the treated plant, the nature of this process was studied.

In the work reported here, an attempt has been made to assess quantitatively the influence of a number of factors on penetration, viz., added surfactant, stage of plant growth, adaxial and abaxial leaf surface, relative humidity, temperature, concentration of herbicide solution, and the removal of cuticular waxes.

LITERATURE REVIEW

Among the several requisite processes involved in the satisfactory performance of a foliar applied herbicide, penetration of the leaf surfaces is the first step after contact with and retention by the foliage. At the outset a distinction must be drawn between the terms absorption and penetration of herbicides. Sargent (55) considered foliar absorption as consisting of a chain of events beginning with the entry of an externally applied substance into the leaf and continuing with its movement within, terminating in its export from the leaf. Penetration, on the other hand, is only that portion of the absorption process by which the externally applied substance moves through the outermost plant surface, prior to its being metabolized at the sites of entry or moved within the plant (55).

A knowledge of the structure and composition of leaf surfaces is essential to an understanding of their effect on herbicidal penetration through these surfaces. The literature dealing with plant surfaces is extensive. A comprehensive review of all facets is beyond the scope of the present discussion, which is intended mainly as a summary of the status of knowledge concerning the physical and chemical nature of leaf surfaces in relation to penetration.

Chemical Nature of Leaf Surfaces:

The outermost layer, lying over the epidermal wall of the leaf surface, is a more or less continuous but non-uniform layer called cuticle (49, 51). The term 'cuticular layer' refers to the semilipoidal lamellae of the surface covering that have become impregnated with wax and cutin. It may be referred to as cutinized cell wall and includes also pectin

and cellulose of epidermal walls. In 1948, Frey-Wyssling (27) summarized the chemical nature of cutinized plant cell walls; they are composed of four rather distinct substances or groups of substances. These are: cutin, waxes, pectin and cellulose. Each constituent imparts its own chemical and physical properties to the plant surface layer and each of these four major components will be discussed briefly.

Cutin:

Cutin is described as a semilipoidal oxidative polymer of unsaturated long-chain fatty acids and alcohols. It is insoluble in most organic solvents and apparently constitutes the matrix of the cuticle. When exposed to water, cutin takes on a negative charge through dissociation of exposed terminal groups containing unsaturated linkages and carboxyl groups. As a result, it tends to attract cations and repel anions. Cutin has both polar and apolar properties; it is semilipoidal but also semipolar. Because of its many polar groups, cutin may absorb water and swell appreciably (65).

Waxes:

The petroleum ether-soluble mixture of saturated lipid substances embedded in the cuticular layers is called cuticular wax (20, 45, 58). Surface wax refers to the irregular and extruded wax rodlets which, under the electronmicroscope, give the outside of a leaf the appearance of a stubble beard (20, 45, 53, 57). Cuticular waxes consist of short, rod-shaped molecules without reactive end groups; being relatively inert, they are unable to polymerize and are of low molecular weight (45, 60).

The waxy rodlets of leaves forming a bloom may prevent contact of

a spray droplet with a leaf surface. Waxy leaf surfaces normally are readily wetted by oils or by aqueous spray solutions containing a suitable surfactant. Surface wax deposits may interfere appreciably with wetting but little with penetration provided that good surface contact is ensured. Despite adequate external wetting, however, internal cuticular wax can still constitute a serious barrier to penetration of aqueous substances (11, 24, 65).

Pectin:

Pectin consists of long-chain polygalacturonic acid molecules having exposed carboxyl groups. These substances occur in an amorphous state and are responsible in large measure for the water-holding capacity of cell walls. Thus, both the pectic layer and the cellulose layer are hydrophilic or polar.

Cellulose:

This structural component is composed of long-chain molecules that are relatively stable. Cellulose, because of its microfibrillar organization, imparts tensile strength and elasticity.

The cuticle of the epidermal cell wall is readily permeable to lipoidal substances and fat-soluble compounds, while the pectin and cellulose phases are readily permeable to water and polar solutes. The combination of the four makes up a unique layer, the outer epidermal wall of the plant foliage.

Physical Nature of Leaf Surfaces:

The cuticle exhibits not only chemical variability, both within and between plants, but also variability in distribution over the leaf

surface, in continuity and degree of perforation, in thickness on various parts of a single leaf and in physical properties (11, 41, 65). Cuticle is usually thicker on the upper than on the lower surfaces (32, 33, 65).

Variation in structure of cuticle between plant species has been reported by a number of investigators (11, 45, 58, 60). In general, succulents such as cacti have a thick cuticle, trees often have a medium to thick cuticle, while herbs vary widely. Most herbs that characteristically grow in shade have a thin cuticle. Differences in surface wax pattern suggest both physical and chemical differences in wax extruded by various species (45, 57).

Although the cuticle is often viewed as a continuous layer over the surface of the plant leaves, much evidence indicates that it varies widely in permeability (11). As would be expected, cracks and perforations increase cuticular permeability (47).

In most plant leaves the wax coating and the cuticle thickness increase with age (45, 57, 60). Skoss (60) found, in Nicotiana glauca and Hedera helix, that the deposition of cuticle is continuous until the leaf reaches morphological maturity. After this no further deposition occurs. Also, the cutin layer of young cuticle is probably more hydrophilic than that of older cuticle, since wax impregnation increases with age (45, 60).

Clearly, cuticle is the first barrier which foliar-applied chemicals must pass. Even when spray solutions enter through the stomata, their active ingredient must still pass the cutin layer which lines the sub-stomatal cavities.

Polar vs. Apolar Routes Across the Cuticle:

Since both polar ions and nonpolar fat-soluble molecules penetrate

the cuticle, both a polar or aqueous route and a nonpolar or lipoid route are assumed to exist in cuticular penetration (11, 24, 55, 65). Several investigators have considered the possibility that water-soluble compounds may follow an aqueous route, and the oil-soluble substances a lipoid route (11, 24, 65), Pallas (48) suggested that an organic molecule such as 2,4-D follows a lipoidal pathway into the leaf, and that an inorganic ion such as phosphate follows an aqueous pathway.

Factors Influencing Penetration:

Currier and Dybing (13) classified the major factors influencing foliar penetration of herbicides into four groups viz. plant factors, environmental factors, composition of spray solution and method of application. A brief discussion of some of these factors is presented below:

Stage of Plant Growth: A decrease in penetration with increasing age of the leaf has been observed by a number of investigators (11, 13, 26, 56, 66). Schieferstein and Loomis (58) observed that the rate of penetration of 2,4-D through ivy cuticle varied with age. The cuticle usually grows thicker, and becomes harder to penetrate by herbicidal solutions during the growing season (26, 58). Relative penetration rates of different cuticle thicknesses indicated, however, that thickness was not the only factor involved (11). The relative abundance of cutin, waxes, and other minor constituents, and their order and arrangement of deposition are undoubtedly important and can be expected to vary among species and with changes in environment.

Foliar sprays of 2,4-D and 2,4,5-T have controlled poplar growth most effectively if they were applied during the early part of the

growing season, as soon as the leaves were fully expanded (26). Later treatments were progressively less effective. Dalrymple and Basler (16) concluded that the absorption of 2,4,5-T in leaves of blackjack oak was highest in early spring and decreased during June. Then there was a gradual increase in the rate of absorption which continued until the middle of August. At this time absorption began a second decrease which continued until the end of the growing season. A decrease in the rate of absorption may be responsible for the low degree of killing of blackjack oak in the later periods of the growing season. These authors concluded, however, that translocation was more important in this instance, since it decreased to a greater extent than did penetration.

Mechanical damage of the leaf surface has been shown to affect its penetration behavior (38, 41, 66). Thus increased penetration associated with leaf necrosis (38), with attack by aphids (38), appearance of cracks in cuticle (11) and artificial breakdown of the cutinized layer of the leaf (66), is not surprising.

Time-Course of Penetration: If it is assumed that penetration occurs by diffusion, the amount of herbicide that would penetrate a leaf surface would depend on: (a) the concentration of the chemical in the solution applied and (b) the length of time the dissolved herbicide is in contact with the leaf. Thus, the rate of penetration is usually greatest for the first few hours with a sharp decrease in later periods (8, 31, 42, 56). Foy (23) observed that in corn leaves a trace amount of dalapon was absorbed almost instantaneously, especially when surfactant was added, and that penetration continued for a period of three hours. The drops usually dried within thirty minutes. Holly (38) concluded that the amount of chlorophenoxyacetic acid herbicides that could be

washed from the leaf surface at various intervals after application, indicated a high rate of penetration for the first few hours, followed by a decrease after 12 hours. This general pattern occurred with several species, though the duration of the initial high rate of penetration varied and was as little as three hours in certain experiments on oats.

Penetration from Adaxial and Abaxial Leaf Surfaces: Marked differences in the rate and amount of penetration by chemicals, including herbicides, through adaxial and abaxial surfaces of the leaf have been shown (7, 14, 19, 33, 56). Two important reasons for these differences can be cited. In the first place, the presence or absence of stomata and their relative number on the two surfaces of the leaf is important. Stomatal penetration, if it occurs, is thought to be by mass-flow as opposed to a diffusion process in cuticular penetration (11, 13, 14, 19). In species where leaves have stomata only on the abaxial surface, most of the studies have shown that penetration is greater through the abaxial surface than through the adaxial surface. Secondly, the cuticle is considered to be thicker on the adaxial surface than on the abaxial surface. It should be pointed out, however, that herbicide solutions entering the leaf via the stomata still encounter a so-called internal cuticle lining the sub-stomatal cavity (65). This layer is normally thinner than the external cuticle but similar in properties (60).

Relative Humidity: High humidity in the atmosphere around the leaf has been found to enhance the penetration of foliar applied herbicides. The accumulated evidence shows that high humidity increased the absorption of foliar applied herbicides and growth regulators such as atrazine,

2,4-D, dalapon, amitrole, alar (B-9) and maleic hydrazide (8, 37, 48, 50, 54, 70). The effect of high humidity on penetration may be explained in several ways. For instance, relative humidity may influence the rate of drying of the treatment droplets and thus affect both the concentration in the droplet and the length of liquid-leaf contact. It may influence the degree of hydration of the cuticle and hence regulate the permeability of this barrier (24, 65). It could modify the behavior of stomata and thus differentiate between a diffusional as compared with a mass-flow of herbicide into the leaf.

Temperature: The rate and amount of penetration of a number of organic compounds into leaves have been found to be affected by temperature. In general, an increase in temperature, within physiological limits, results in increased penetration (3, 8, 31, 42, 44, 48). The amount of absorption of the NH_4^- salt of 2,4-D in red kidney bean plants was positively correlated with temperature over a three-level range (58°F , $79-82^\circ\text{F}$ and $86-92^\circ\text{F}$) (52). On the basis of the relatively high temperature coefficients obtained, a number of investigators have claimed that penetration is metabolically governed (31, 42, 55, 56). Undoubtedly, metabolic processes could exert an indirect influence on penetration rate by influencing cytoplasmic viscosity, accumulation, binding and translocation of the penetrant. Temperature, however, can directly influence the rate of diffusion of herbicides through the cuticle and other lipid-containing membranes. Van Overbeek (65) indicated that fatty molecules oriented in layers as micelles have a very low permeability at low temperatures when the layer nears solidification. At high temperatures, these fatty substances become less viscous and offer less resistance to the passage of diffusates. Briggs et al. (5), in a

consideration of the oriented lipoidal molecules system, state that the kinetic energy which a lipophilic molecule must acquire before it is able to break the bonds between the oriented molecules is relatively large and is likely to yield temperature coefficients in excess of 2 for diffusion.

Removal of Cuticular Waxes: The wax components of the cuticular membrane play a significant role in governing the sorptive properties of the leaf cuticle of various plants (47, 57). Removal of surface waxes from the cuticle has been found to increase the rate and extent of cuticular penetration (6, 17, 59). Bukovac and Norris (6) recently found that sorption of naphthalene acetic acid by the isolated cuticle of pear leaf (Pyrus communis L.) is increased on removal of surface wax. Only small further increases resulted from subsequent extraction of embedded waxes. Similarly, Darlington and Barry (17) concluded that the permeability of isolated cuticle of apricot to acetamide can be increased markedly by prior soaking of the cuticle disks in chloroform. Further, Ennis et al. (12) obtained complete droplet retention when the waxy layer of the leaves of soybean and cabbage was removed by rubbing the surface slightly with a finger.

The cuticular waxes are readily removed from leaves by treatment with a suitable solvent, but it is necessary to ensure that the leaf tissues do not die. Chloroform is a good solvent to remove surface waxes from the leaf cuticle (6, 17, 59). Silva Fernandes et al. (59), for example, removed the surface waxes from leaves by immersing them individually, with agitation, for 5-10 seconds, in successive portions of chloroform at room temperature.

Surfactant Enhancement of Foliar Penetration:

Surfactants are defined as substances capable of altering the energy relationships at surfaces or interfaces, thereby reducing interfacial tension (4, 24). Behrens (4) has described the principal physical and chemical properties of surfactants in general. He concluded that the presence of two dissimilar chemical groups - hydrophilic and lipophilic - in a single molecule is responsible for their surface - active nature.

It has long been recognized that surfactants may facilitate and accentuate the emulsifying, dispersing, spreading, wetting, solubilizing and/or other surface-modifying properties of herbicidal formulations and may bring about enhancement of penetration and herbicidal action (3, 4, 14, 19, 25, 39, 40, 56, 62). The most efficient surfactants were reported to increase plant response to 2,4-D about fourteen-fold (69). While the primary purpose of a surfactant added to a herbicide is to promote wetting and coverage of the leaf surfaces by reducing surface tensions of aqueous systems (13, 24, 55, 62), the unique hydrophilic-lipophilic properties of these additives (4) have suggested that they may be capable of more than this one function. For the present discussion it is sufficient to say that surfactants act, perhaps primarily, by virtue of their combined polar and apolar properties in the same molecules, rendering compatible two phases (e.g. lipoidal and nonlipoidal substances) which were otherwise incompatible. Some surfactants probably become solubilized in the cuticle, thus causing a loosening or swelling of the cuticular architecture and thereby enhancing penetration (24, 39, 40, 65).

Herbicide Formulations: The influence of slight structural changes on activity of growth regulators is striking. Penetration may be a

controlling factor in these changes but there is little direct evidence that this is so (38, 65).

In general apolar or slightly polar molecules are thought to enter leaves more readily than polar molecules (9,10,65). Penetration of growth regulators may be improved when the molecule exhibits the proper polar-apolar balance. According to van Overbeek (65), ester formulations of 2,4-D that have both water - and fat - solubility are more effective than the polar acid and salt formulations.

MATERIALS AND METHODS

Experiments were conducted with detached aspen and balsam poplar leaves collected from mature trees outside (south bank of the North Saskatchewan river in Edmonton) or from young greenhouse-grown trees, and with intact plants of Canada thistle. The leaves are referred to as outside and greenhouse leaves, respectively, in the text.

Plant Culture in the Greenhouse:

Aspen Poplar: In the greenhouse the aspen poplar plants were grown by a technique similar to that of Eliasson (22). Root cuttings about 15 cm long, taken from two aspen poplar trees at the Woodbend field station, 25 miles southwest of Edmonton, were planted in wooden flats with moist vermiculite, in June, 1967. The temperature in the greenhouse was maintained at approximately 20°C during the winter. During the summer, however, temperatures in excess of 35°C were occasionally experienced. Supplementary lighting was given during the winter months to keep the daylength at 16 hours. When the shoots produced by the root cuttings were 10 - 12 cm high, they were transplanted to 15 cm plastic pots with a 3:1 mixture of greenhouse soil (three parts of black loam to one part of sand and one part peat) and vermiculite. Leaves were collected from these plants when they were 1 1/2 - 2 feet high and had a good number of leaves. Considerable difficulty arose in the culture of aspen poplar plants in the greenhouse because of low survival potential of the shoots produced by the root cuttings.

Balsam Poplar: Balsam poplar plants were grown in the greenhouse from stem cuttings of about 15 cm length and with 2-3 buds each. To promote rooting, their tips were dipped in a commercial powder formulation of 3-

indolylbutyric acid (trade name Seradix B) before planting in the soil.

The leaves from these plants were collected for treatment after they had attained a good growth. Growing conditions were similar to those described for culture of aspen poplar plants.

Canada Thistle: To avoid genetic variability the Canada thistle plants used in these experiments were all vegetatively propagated from a single plant. Each plant was grown from a single creeping root section about 6 cm long. The plants were grown in 15 cm plastic pots and treated usually 5-6 weeks after planting when they were 10-14 cm high. The greenhouse conditions were usually the same as described for the culture of aspen poplar plants.

Experimental Materials: The herbicides included in this work were derivatives of picloram (4-amino-3, 5, 6-trichloropicolinic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid). Commercial formulations used were: potassium salt of picloram (trade name Tordon 22k), dimethyl amine of 2,4-D (trade name Weedar 80), and ethyl ester of 2,4-D (trade name Weedone). The sources of the herbicides are listed in the Appendix (Table 1). Atlox 210, a non-ionic blended surfactant, containing polysorbate, mono- and diglycerides, butylated hydroxyanisole, butylated hydroxytoluene and propylene glycol in a water-isopropanol medium, was added to the herbicide solutions in some cases.

Aspen poplar leaves were collected outside during four growth periods: June, July, August, September. Initial bud opening occurred near May 10, 1967. The leaves attained maximum size in about one and half months after initial bud opening. To reduce variability the leaves were collected from one-year-old branches of four selected trees throughout the investigation. Leaves were collected between 8 and 9 a.m., on

bright sunny days with outside temperatures ranging from 75°-85°F. The detached leaves were kept turgid during the treatment periods by immersing their petioles in tap water.

High humidity was attained in a closed system allowed to become saturated or nearly so with water vapor. Most of the experiments were conducted under normal laboratory conditions at temperatures of $25.5 \pm 1.5^{\circ}\text{C}$, in diffuse daylight supplemented by fluorescent light. Low temperatures (10°C) and high temperature (40.5°C), whenever required, were attained in a cold room and an incubator, respectively. Waxes were removed from the leaf surfaces in some experiments, by dipping the leaves in chloroform for periods varying from 10 to 240 seconds.

General Application of Treatments: For each treatment three leaves were used. All leaf applications were made in water solution or emulsion. In some cases surfactant (Atlox 210) was added to the herbicide solutions at a final concentration of 1% for poplars and 2% for Canada thistle. Progressive enhancement in herbicidal penetration and activity with an increase in surfactant concentration up to a certain level has been reported in the literature (4, 15, 29, 56). Surfactant, Atlox 210, at 0.25 - 1.0 percent concentration increased the activity of atrazine, diuron, 2,4-D and picloram to a marked extent (15, 29).

Picloram and 2,4-D amine were applied with a microsyringe, in doses of 0.2 mg in 40 or 100 μl of solution. Twenty- μl droplets were placed about 1 cm away from the midvein of poplar leaves. The dose of 2,4-D ester was 0.1 mg in 100 μl emulsion. Droplets of 2,4-D ester were covered with plastic caps to reduce volatility losses. Evaporation losses of the volatile 2,4-D ester from parafilm were estimated by determining residual amounts after varying intervals. In Canada thistle

all herbicide applications were made on the midrib of a leaf, in a lanolin ring.

Spectrophotometric Assay: Spectrophotometric assay has been used to measure the changes in amount of growth regulator remaining on the leaf surfaces after application, and from these measurements calculations have been made of the amount penetrated or absorbed by the leaf (2, 37, 38, 52, 63). In our experiments also the amount of residual picloram or 2,4-D on the leaves was determined spectrophotometrically. The absorption spectra of picloram and 2,4-D in 50% ethanol were determined on a Beckman DK Recording Spectrophotometer. The wavelengths used for determinations of herbicide concentrations were 270 and 283 $m\mu$ for picloram and 2,4-D, respectively. Standard curves for these herbicides were prepared using a series of concentrations ranging from 0 to 60 $\mu\text{g/ml}$.

One to 24 hours after treatment the leaves were washed in 5 ml of 50% ethanol, in a slow continuous flow from a burette. The concentration of residual herbicide in washings was determined in an aliquot of about 3.5 ml, sufficient for a sample vial in the spectrophotometer. The difference between the dose applied and the residual amount of herbicide, after correction for volatility losses, was assumed to be the amount that had penetrated into the leaf.

Radioactive Assay: The amount of penetration into the leaves was also investigated in some cases by determining the radioactivity in extracts of plant material treated with labeled herbicides.

Four 10 μl droplets of water containing a total of 0.1 μc picloram*¹ (C^{14} carboxyl labeled, potassium salt of picloram prepared by mixing together appropriate quantities of labeled and blank formulation, specific activity 1.03 $\mu\text{c/mM}$) were placed on detached aspen and balsam

¹Obtained from Dow Chemical Co., Inc.

poplar leaves in the manner described previously. Three leaves were included in each treatment. In Canada thistle a 40 μ l drop of this solution was applied in a lanolin ring on the midrib of a full-grown leaf. The concentration used was 2.5 μ c/ml (0.59 mg/ml). Butoxy ethyl ester of 2,4-D*²(C¹⁴ - carboxyl labelled) in 50% ethanol was applied to the aspen poplar leaves in two 20 μ l drops containing a total of 0.7 μ c 2,4-D. The concentration used was 1.66 μ c/ml (0.53 mg/ml). Each droplet was covered with a plastic cap sealed to the leaf with lanolin to minimize volatility losses. Volatility losses of 2,4-D*ester from a sheet of parafilm were studied by determining residual material after varying periods.

Determination of Radioactivity in Extracts: The treated leaves were washed with 10 ml of 50% ethanol after periods of 1 to 48 hours. The leaves were then ground for three minutes in about 30 ml of 95% ethanol in a Waring blender. The Canada thistle plants were divided into shoot and root portions for the determinations of radioactivity. Flasks with ground material were kept at room temperature for about 6 hours. The preparations were then filtered through Whatman No. 1 filter paper in a Buchner funnel. The extracts were evaporated to dryness under reduced pressure in a rotary evaporator at 38-40°C and were then brought to a volume of 5 ml by adding 95% ethanol.

Aliquots of 0.5 ml of the concentrated extract were placed on aluminum planchets and dried on a hot plate. To each planchet 50 μ l of 1% Tween 20 was added to ensure even distribution of the extracts. Radioactivity of the extracts was then determined in a Nuclear Chicago

²Obtained from New England Nuclear Corp., Massachusetts through the courtesy of Amchem Products Inc.

Model D-47 gas flow counter with an efficiency for C^{14} of approximately 33% and an average background of 20 CPM. Corrections were made for background and aliquot fraction. A similar procedure was followed for determination of radioactivity in leaf washings.

Autoradiography: Foliar penetration and the distribution of picloram in detached aspen poplar leaves was studied in some cases by autoradiography as described by Crafts and Yamaguchi (12).

The solution of picloram was applied in a manner similar to that mentioned for the radioactive assay. At the end of the treatment period, the residue on the leaf surface was washed off with 10 ml of 50% ethanol. Duplicate leaves were freeze-dried, in a tank equipped with a moisture trapping flask placed in the vacuum line and bathed in a mixture of acetone and dry ice. The drying process was completed in approximately three days. The dried leaves were humidified, mounted and then autoradiographed for three weeks using Ansco. non-screen safety X-ray film.

RESULTS

Spectrophotometric Assay of Herbicides: Figure 1 presents the absorption spectra obtained for picloram, 2,4-D amine, and 2,4-D ester in 50% ethanol (20 $\mu\text{g/ml}$). The wavelengths used for determinations of herbicide concentrations were 270 and 283 $\text{m}\mu$ for picloram and 2,4-D, respectively. At these wavelengths standard curves for the three herbicides were prepared (Fig. 2). These curves illustrate the linear relationship between optical density and concentration for solutions of each of the chemicals in the concentration range used.

Preliminary studies on recovery of herbicides from the leaf surfaces gave a recovery of 98.5 ± 1.5 percent when the leaf was washed with 5 ml of 50% ethanol immediately after treatment, and the concentration determined spectrophotometrically.

PENETRATION IN ASPEN POPLAR:

Influence of Added Surfactant and Relative Humidity:

A. Adaxial Leaf Surfaces: Leaves for this series of experiments were collected 25 days after initial bud opening. Dosage for both picloram and 2,4-D amine was 200 μg per leaf. Results of leaf surface residue determinations (volatility losses for picloram and 2,4-D amine were negligible and those of 2,4-D ester were subtracted to get the net amount of penetration) 1 to 24 hours after droplet application indicated that added surfactant markedly increased the penetration of both picloram and 2,4-D amine (Figs. 3 and 4). The addition of surfactant resulted in approximately doubling the amount of herbicide penetrated in 24 hours in all cases except for 2,4-D amine under high humidity. Here the increase was only 61 percent. The addition of surfactant was more effective under low

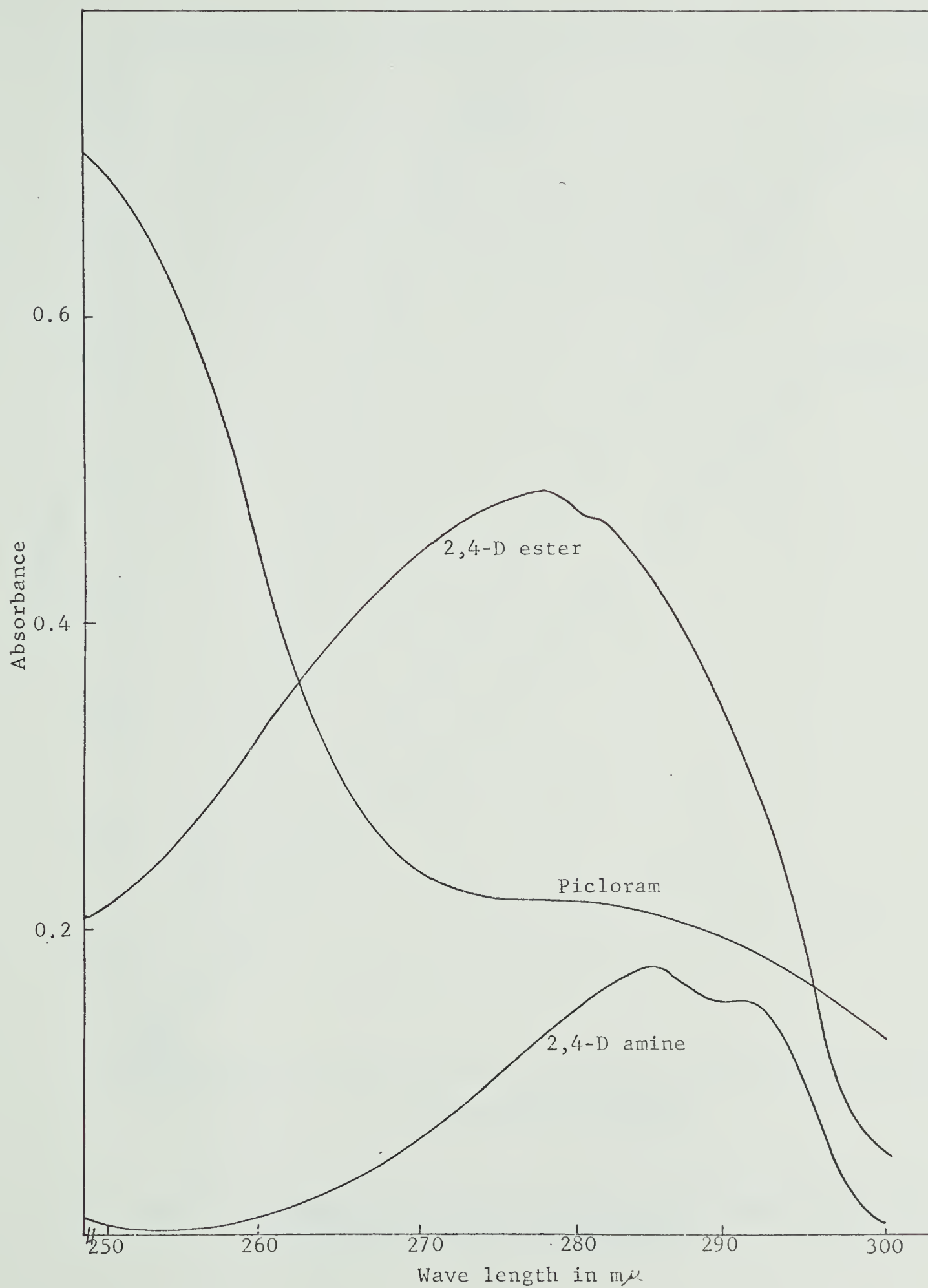


Fig. 1. Absorption spectra of 2,4-D (amine and ester) and picloram ($20\mu\text{g/ml}$) in 50% ethanol.

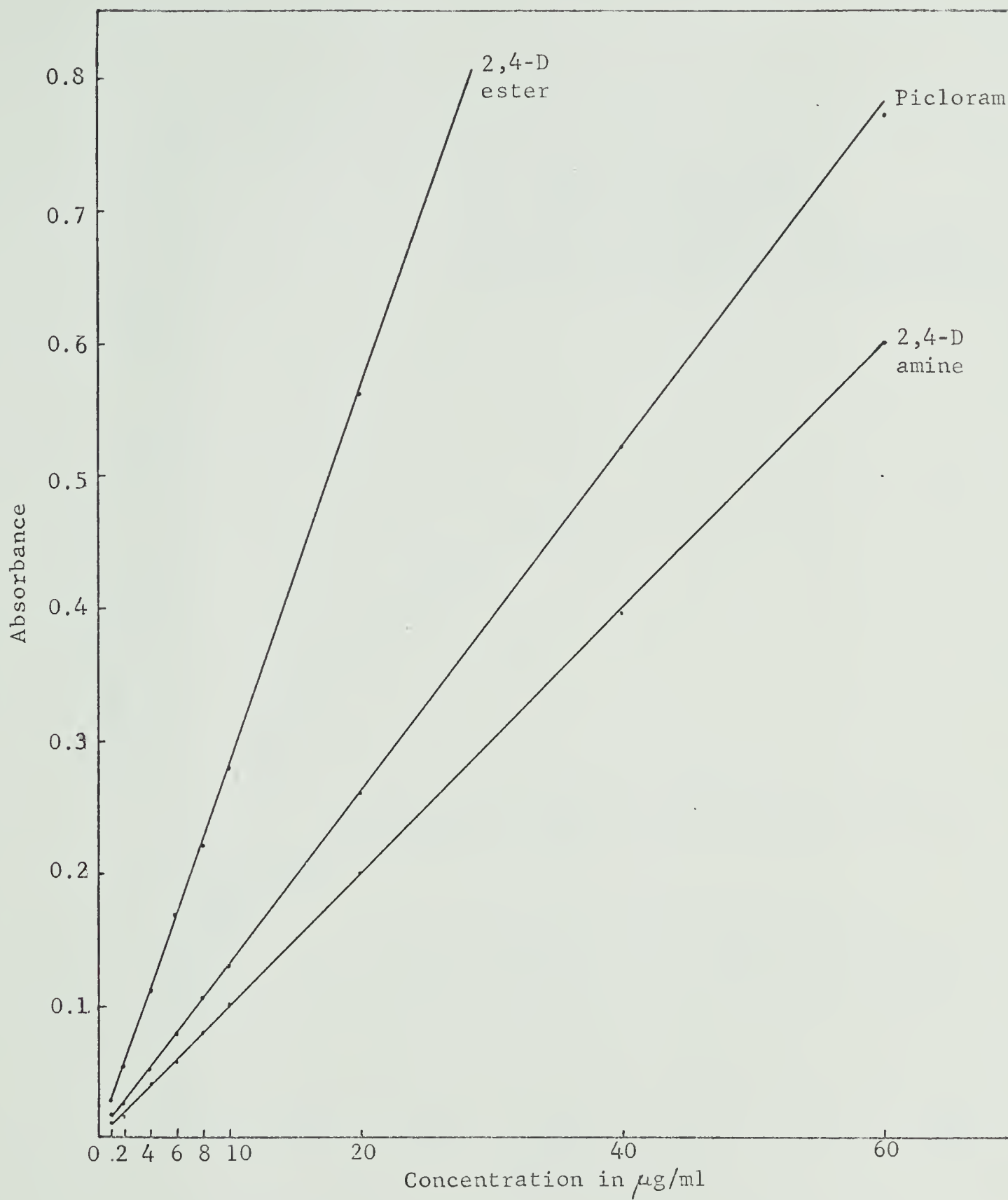


Fig. 2. Standard curves for 2,4-D (amine and ester) and picloram at concentrations of 1 to 60 $\mu\text{g/ml}$.

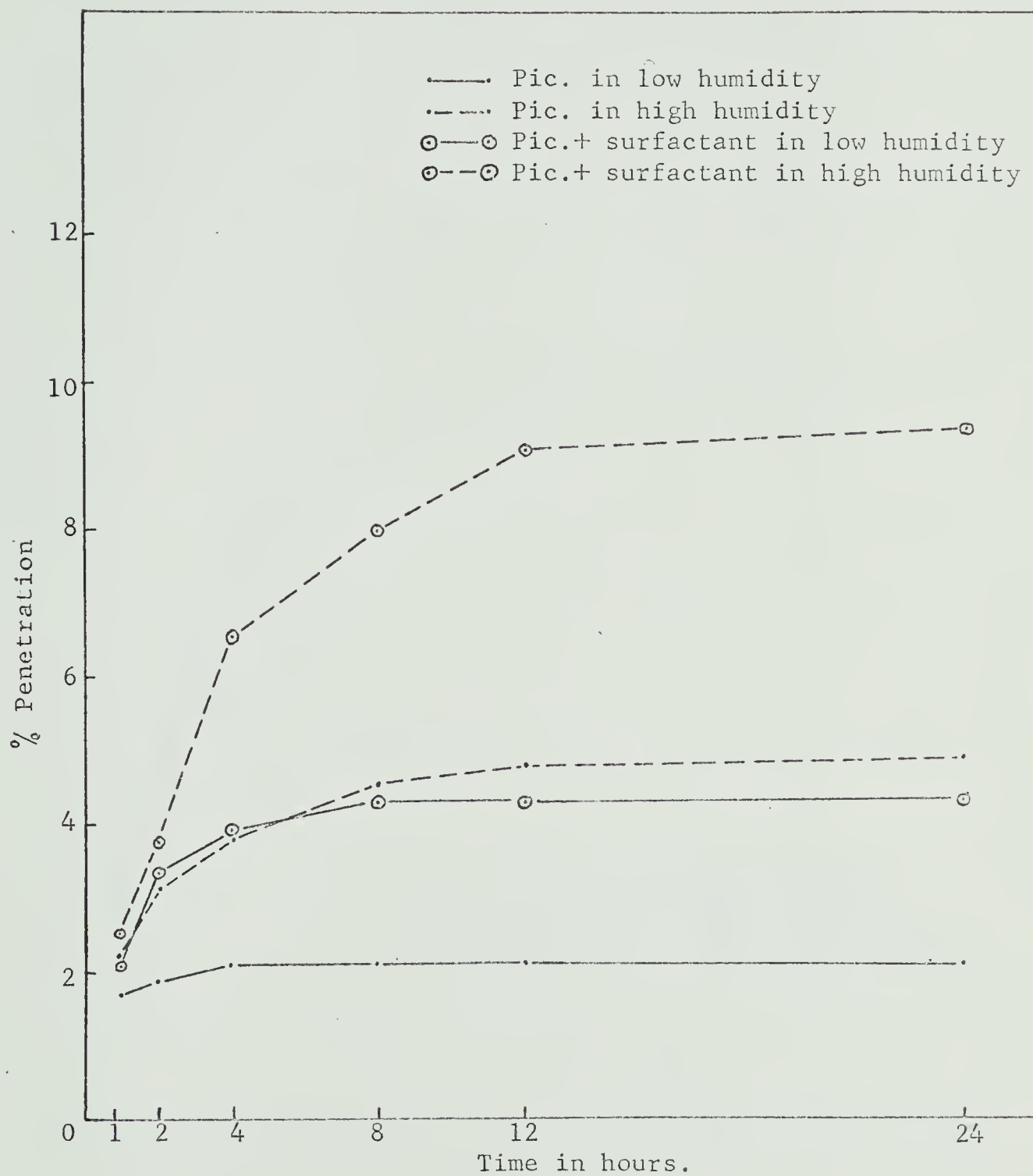


Fig. 3. Penetration of picloram (200 µg) with and without added surfactant (1% Atlox 210) from the adaxial surface of aspen Poplar leaves under low and high humidity.

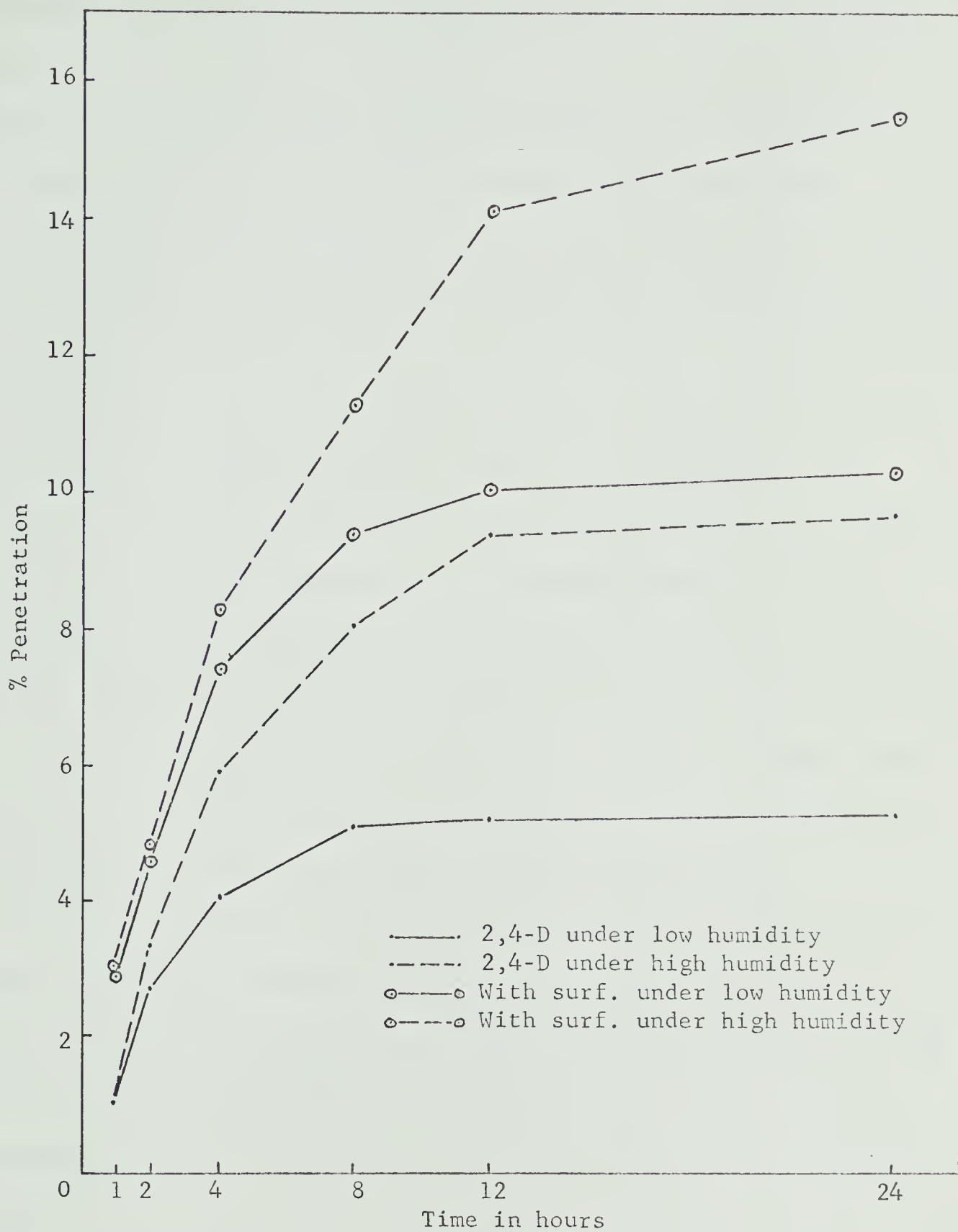


Fig. 4. Penetration of 2,4-D amine (200 µg) with and without added surfactant (1% Atlox 210) from the adaxial surface of aspen Poplar leaves, under low and high humidity.

humidity than under high humidity conditions for both picloram and 2,4-D amine.

Under low humidity without added surfactant, crystal formation of the chemical was observed on the treated spots 3 to 4 hours after application. With added surfactant, on the other hand, crystal formation did not occur until 8 to 10 hours after application. Similar observations were made under high humidity, where crystallization was noticed after 10 to 14 hours without surfactant, and with surfactant almost no crystallization occurred even after 24 hours. Addition of surfactant to the herbicide solutions thus prevented or postponed crystallization of the chemical in the treatment droplets. By maintaining the spray deposit in a fluid state and holding water at the surface of the leaf, surfactant may provide conditions suitable for increased penetration of picloram and 2,4-D. This is in accord with the conclusions of Hughes and Freed (39), who considered that surfactants may act as humectants by retarding drying out of the solution to provide a longer period of penetration.

Penetration of picloram and 2,4-D amine, with or without surfactant, was greater under high humidity than under low humidity conditions (Figs. 3 and 4). Increases for picloram were about double those for 2,4-D, both with added surfactant and without. For both herbicides the response to high humidity was greater in the absence than in the presence of added surfactant.

Under low humidity the droplets of herbicide solution dried out to a white crystalline powder on the surface of the treated spots. The prolonged liquid contact under high humidity undoubtedly plays a role in bringing about increased penetration. It should also be noted that under high humidity penetration continued to occur to some extent between 12

and 24 hours after treatment, especially where surfactant was added to the solution, even though the droplets dried between 9 and 11 hours after application (Figs. 3 and 4). Under low humidity, on the other hand, no appreciable penetration occurred after the droplets had dried except where surfactant was added to the solution.

The influence of added surfactant and high humidity was also investigated by using labeled picloram. Very little picloram* penetrated except in the presence of added surfactant, under high humidity.

To investigate if high humidity increases the penetration of picloram* through its retarding effect upon drying of the droplets, the treated area was rewetted with 10 μ l quantities of distilled water at 1 hour intervals for a period of 8 hours under low humidity. The droplets were wet for 8 hours under rewetted conditions, and for 15 hours under high humidity conditions. Penetration of picloram* (with added surfactant) 48 hours after treatment under rewetted conditions did not reach the level of penetration under high humidity (Table I). It seems, therefore, that uptake is quite closely correlated with the time of exposure to the picloram* in solution. However, the differences in penetration under the two conditions after 8 hours indicate that increased penetration under high humidity cannot be attributed entirely to the rate of drying of the droplets. Humidity may have some indirect effects in modifying the behavior of the cuticle on leaf surfaces and hence may regulate the permeability of this barrier.

Table I. Penetration of picloram* with added surfactant (1%), as a percentage of the dose applied ($0.1 \mu\text{c}$ in $40 \mu\text{l}$), under low humidity (droplets rewetted for 8 hours) and high humidity.

Time in hours	Low humidity (rewetted)	High humidity
4	0.6	0.7
8	1.4	1.5
12	2.0	2.7
24	3.0	5.6
48	3.0	7.3

There is little doubt that the longer the leaf is exposed to the herbicide treatment, the greater the quantity absorbed (Figs. 3 and 4). There is a definite indication of a high rate of penetration for the first few hours. Under high humidity penetration was rapid up to 12 hours, and then gradually decreased. Under low humidity, on the other hand, the duration of the initial high rate of penetration was as little as 4 hours. The rate of droplet drying, and thus the length of time the dissolved herbicide is in contact with the leaf, may be considered an important factor in determining the rate and extent of penetration of picloram and 2,4-D into the leaves.

The results also indicated that in each treatment the rate of penetration of 2,4-D amine was almost double that of picloram.

B. Abaxial Leaf Surfaces: In aspen poplar leaves stomata were found to occur only on the abaxial surfaces. Examination of leaf sections in a light microscope indicated that at the time of treatments the stomata were mostly open. Both added surfactant and high humidity drastically increased the penetration of picloram and 2,4-D from the abaxial leaf surfaces

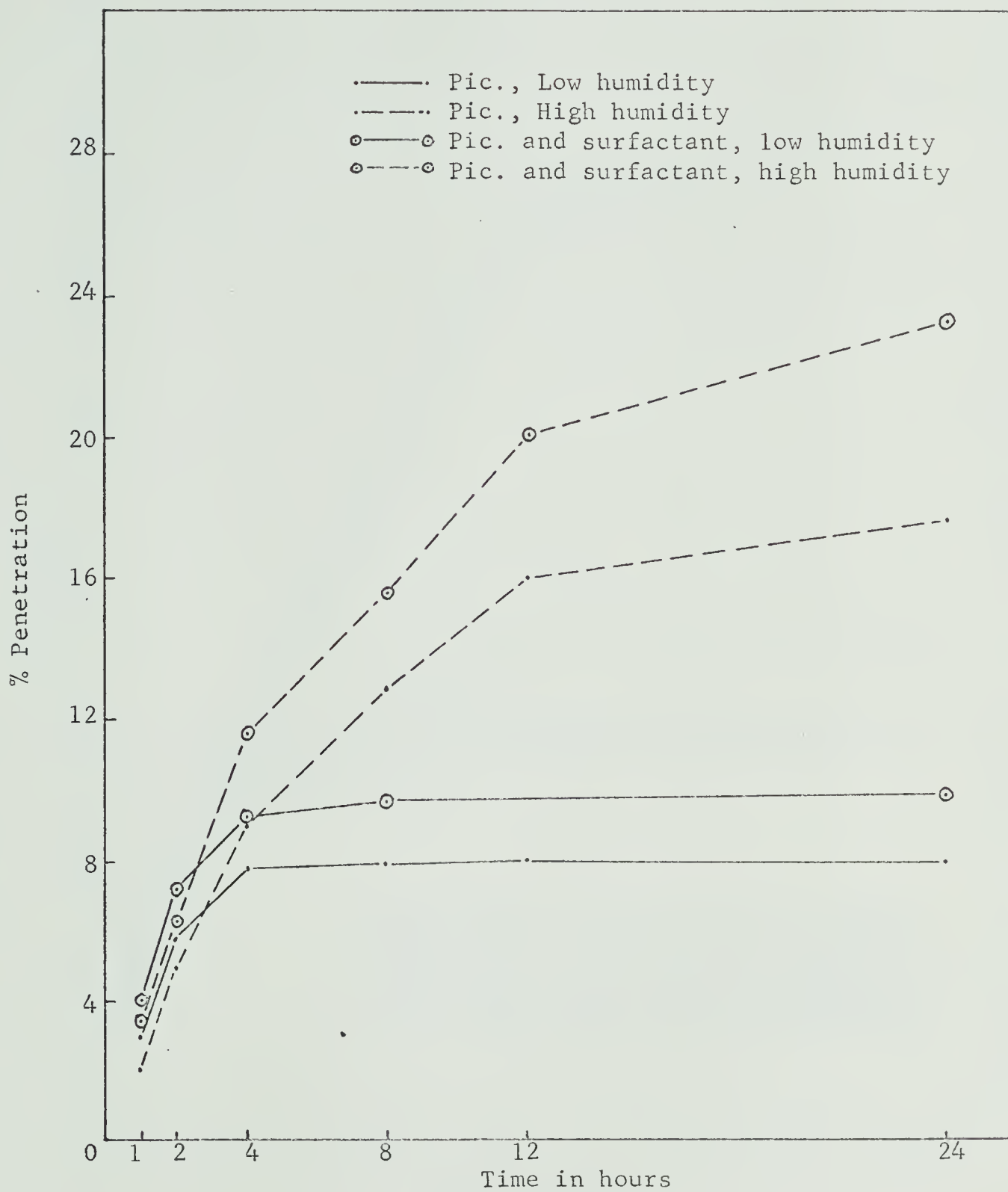


Fig. 5. Penetration of picloram (200 µg) with and without added surfactant (1% Atlox 210) from the abaxial surface of aspen poplar leaves under low and high humidity.

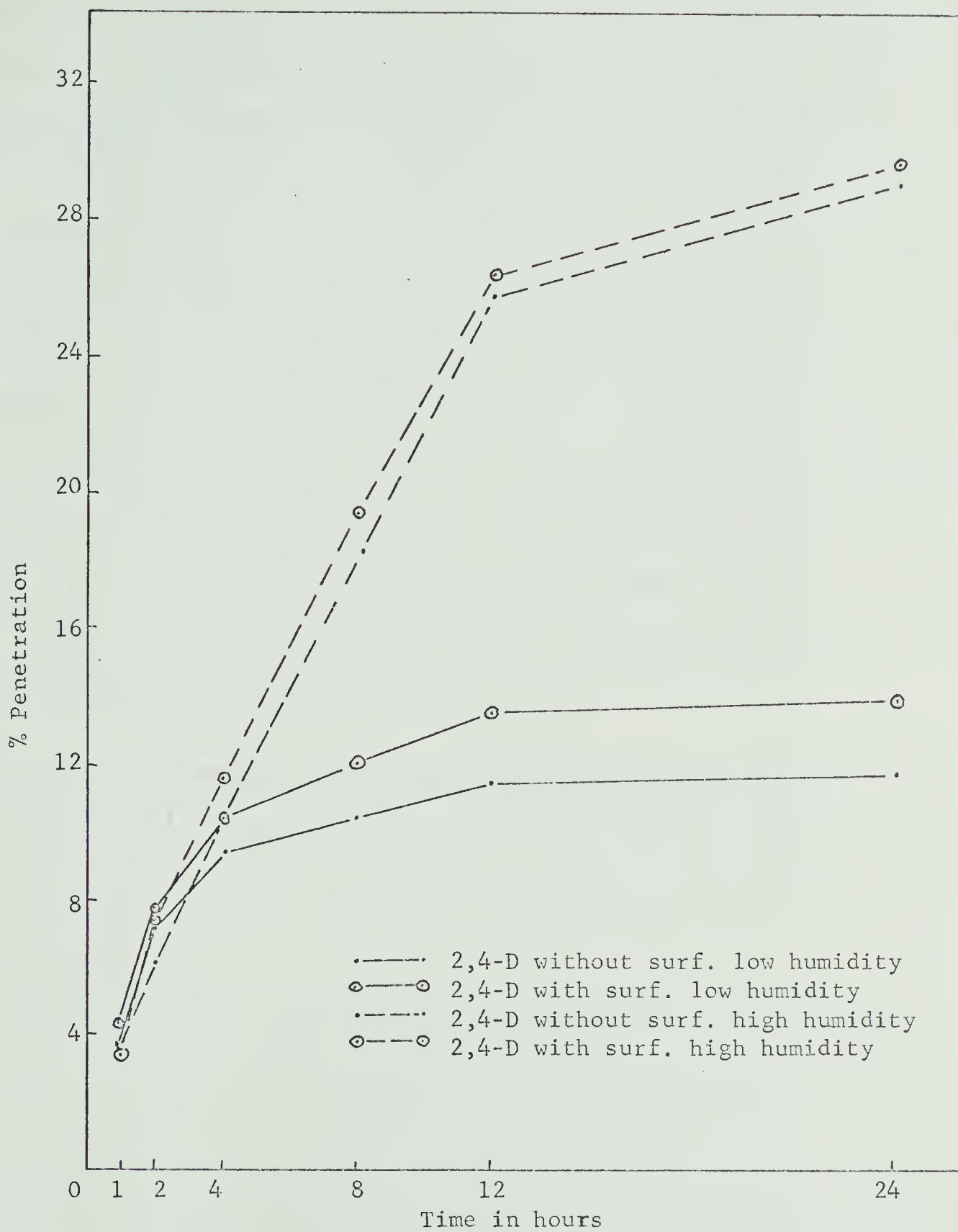


Fig. 6. Penetration of 2,4-D amine (200 µg) with and without added surfactant (1% Atlox 210) from the abaxial surface of aspen poplar leaves under low and high humidity.

(Figs. 5 and 6). No marked differences were observed during the first two hours, but differences became very significant after that. Added surfactant increased penetration of 2,4-D amine under low humidity, but had little effect under high humidity (Fig. 6). In contrast to this, addition of surfactant to picloram solution was equally effective in enhancing penetration under both low and high humidity (Fig. 5).

The rate and amount of penetration of picloram and 2,4-D amine were considerably greater from the abaxial surfaces of the leaf than from adaxial surfaces (Figs. 3, 4, 5 and 6). Under both low and high humidity, and with and without added surfactant, abaxial penetration was two to four times the adaxial rate for picloram, and almost doubled for 2,4-D amine. These results correspond with reports of similar observations in the literature (3, 14, 19, 33, 56).

Penetration of 2,4-D Ester from Adaxial and Abaxial Leaf Surfaces: In experiments with 2,4-D ester, surfactant (Atlox 210) at a concentration of 1% was included in all the herbicide preparations, in order to obtain a stable emulsion.

Volatility losses of 2,4-D ester from parafilm, from droplets of emulsion covered with plastic caps ~~and lanolin~~, were determined after various intervals. Losses of up to 56 percent of the total dose applied were observed, over a 24-hour period (Table II). The volatility losses at different periods of time were subtracted from apparent penetration losses of 2,4-D ester from the leaf surfaces to get the net amount of herbicide penetrated into the leaves.

Table II. Volatility losses of 2,4-D ester from parafilm, as a percentage of the total dose applied ($100\ \mu\text{g}$ in $40\ \mu\text{l}$), under low and high humidity.

Time in hours	Low humidity	High humidity
1	18.6	18.4
2	32.4	32.3
4	41.5	41.6
8	52.6	52.6
12	56.2	56.2
24	56.4	56.3

The rate and amount of penetration of 2,4-D ester did not differ greatly between adaxial and abaxial leaf surfaces or between low and high humidity (Table III). There was, however, a tendency towards greater penetration from abaxial leaf surfaces and under high humidity than from adaxial surfaces and under low humidity. As the herbicide droplets under both low and high humidity were covered, one should not expect much difference in penetration due to variation in humidity conditions around the leaves. Further, it appears that 2,4-D ester can penetrate the leaf cuticle on the adaxial surface and the stomata and leaf cuticle on the abaxial surface about equally well.

Table III. Penetration of 2,4-D ester, as a percentage of the dose applied ($100\ \mu\text{g}$ in $40\ \mu\text{l}$), from the adaxial and abaxial surfaces of aspen poplar leaves under low and high humidity.

Time in hours	Adaxial surface		Abaxial surface	
	Low humidity	High humidity	Low humidity	High humidity
2	5.7	6.2	7.9	8.3
4	15.7	16.8	18.2	18.3
8	25.3	25.3	27.6	27.7
12	31.4	31.4	33.8	33.8
24	32.8	33.0	33.8	34.6

Chlorosis and tissue browning on the 2,4-D ester treated spots was observed on both adaxial and abaxial surface of the leaves (Fig. 7).

To investigate the influence of added surfactant on penetration of 2,4-D ester an experiment was conducted with labeled 2,4-D (butoxy ethanol ester). The treatment solution was prepared in 50% ethanol. Surfactant (Atlox 210) was added at a concentration of 1%. Volatility losses of this formulation from parafilm, from droplets of solution covered with plastic caps ~~and lanolin~~, under low humidity, were reasonably low: about 16 percent over a period of 24 hours. Added surfactant under low humidity enhanced the penetration of 2,4-D* ester from adaxial surfaces of the leaf by about 20 percent in a period of 24 hours (Table IV). The rate and amount of penetration of 2,4-D* ester from the adaxial surfaces of the leaf were greater than those of unlabeled 2,4-D ester. This discrepancy might be due to the presence of ethanol, a relatively nonpolar molecule, in the treatment solution. It should be noted that surfactant enhancement of penetration of 2,4-D* ester was much less than that obtained for picloram and 2,4-D amine (Figs. 3 and 4).

Table IV. Penetration of 2,4-D* ester as a percentage of the total dose applied (0.07 μ c in 50 μ l) with and without added surfactant (1%) from the adaxial surface of aspen poplar leaves under low humidity.

Time in hours	Without surfactant	With surfactant
2	5.7	6.0
4	10.6	13.0
8	21.5	26.5
12	34.6	42.6
24	45.9	54.8

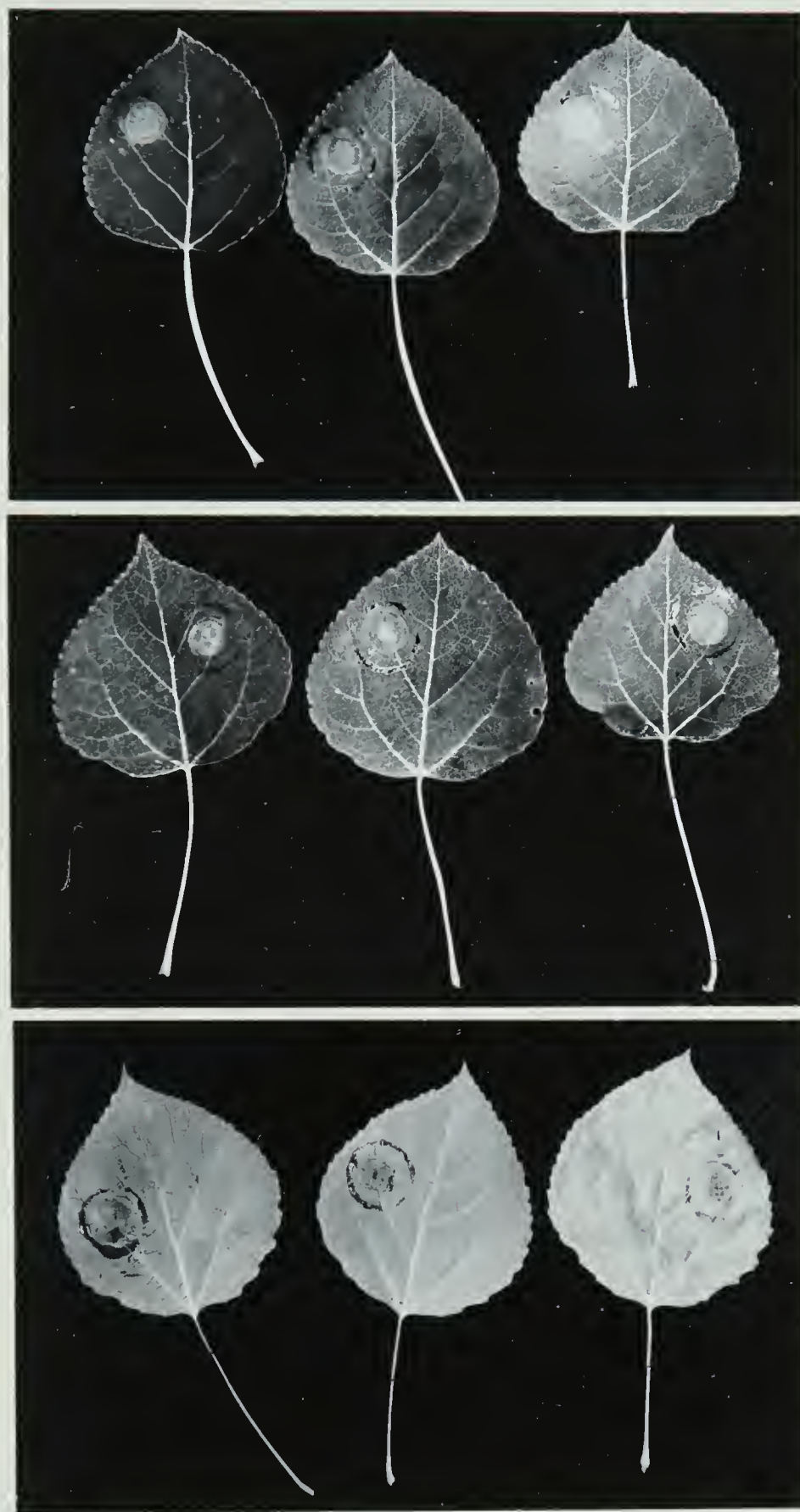


Fig. 7. Aspen poplar leaves showing contact injury (brown colored spots) on the treated area after 12 hours of treatment with 2,4-D ester ($100 \mu\text{g}$ in $40 \mu\text{l}$). Top: Adaxial surface under low humidity. Middle: Adaxial surface under high humidity. Bottom: Abaxial surface under high humidity.

From the results presented so far it can be concluded that there was considerable difference in penetration between picloram, 2,4-D amine and 2,4-D ester. The order of penetration of the three herbicides was: 2,4-D ester > 2,4-D amine > picloram. The greater initial rate and amount of penetration of ester formulation of 2,4-D undoubtedly played a significant role in bringing about faster and more effective killing of foliage of aspen and balsam poplar species in experiments reported by Dai (15).

Influence of Stage of Growth: Penetration of the three herbicides in aspen poplar leaves was studied during four growth periods: June, July, August, September. At each growth stage the influence of added surfactant and relative humidity on penetration from both the adaxial and abaxial leaf surfaces was studied, over a period of 1 to 24 hours after droplet application. Adaxial leaf surfaces were treated in the first week, and abaxial leaf surfaces in the last week of each growth period.

The enhancing effect of added surfactant and high humidity on penetration of herbicides was similar at all four growth stages examined.

Penetration of picloram and 2,4-D amine from adaxial leaf surfaces increased in July and then decreased again in August and September, to a level equal to or lower than that in June (Table V).

Table V. Penetration of three herbicides, as a percentage of the dose applied, with added surfactant (1%) under high humidity after 24 hours of treatment.

Growth periods	Picloram		2,4-D amine		2,4-D ester	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
June	9.4	23.2	15.6	31.5	33.6	34.6
July	15.1	23.4	26.9	32.1	33.5	32.1
August	9.4	20.9	14.3	29.5	32.7	31.7
September	8.8	19.4	14.0	27.6	32.0	32.6

Penetration of 2,4-D ester was influenced but little by growth stage. One would expect a decrease in penetration with leaf age as a result of increased cuticle thickness (11, 45, 56). The increase in penetration observed in July in these studies may be attributed to certain physical changes noticed on the adaxial surface of the leaves. In contrast to the smooth and uniform surface in June (photographed June 5) the leaf surface one month later (July 5) had many cracks and punctures (Fig. 8). These might have developed as a result of changes in the environment or attack by insects and diseases. Juniper (41) suggested that the lateral stretching of the wax layer on the leaf during growth may cause non-uniformity in the surface. This damage to the leaf in the July period may have resulted in increased penetration. A decrease in penetration in August and September may be due to recovery of the leaf surface from such mechanical damage or to increased cuticle thickness with age (leaves had a glazy appearance in August and September). Juniper (41) concluded that some species that are susceptible to mechanical damage to their wax covering also seem to possess powers of recovery during at least the early stages of their development.

Penetration of herbicides from abaxial leaf surfaces did not differ greatly between June and July, chiefly because the leaves for the two series were collected near the end of June and July, respectively, and in both instances showed surface damage consisting of small yellowish punctures. In August and September there was a considerable decrease in penetration. To account for this relationship there seemed to be two possible explanations: (1) Some factor such as cuticle thickness may increasingly limit penetration as the leaf matures; (2) there are a number of sites where penetration of herbicides may occur (i.e. stomata)

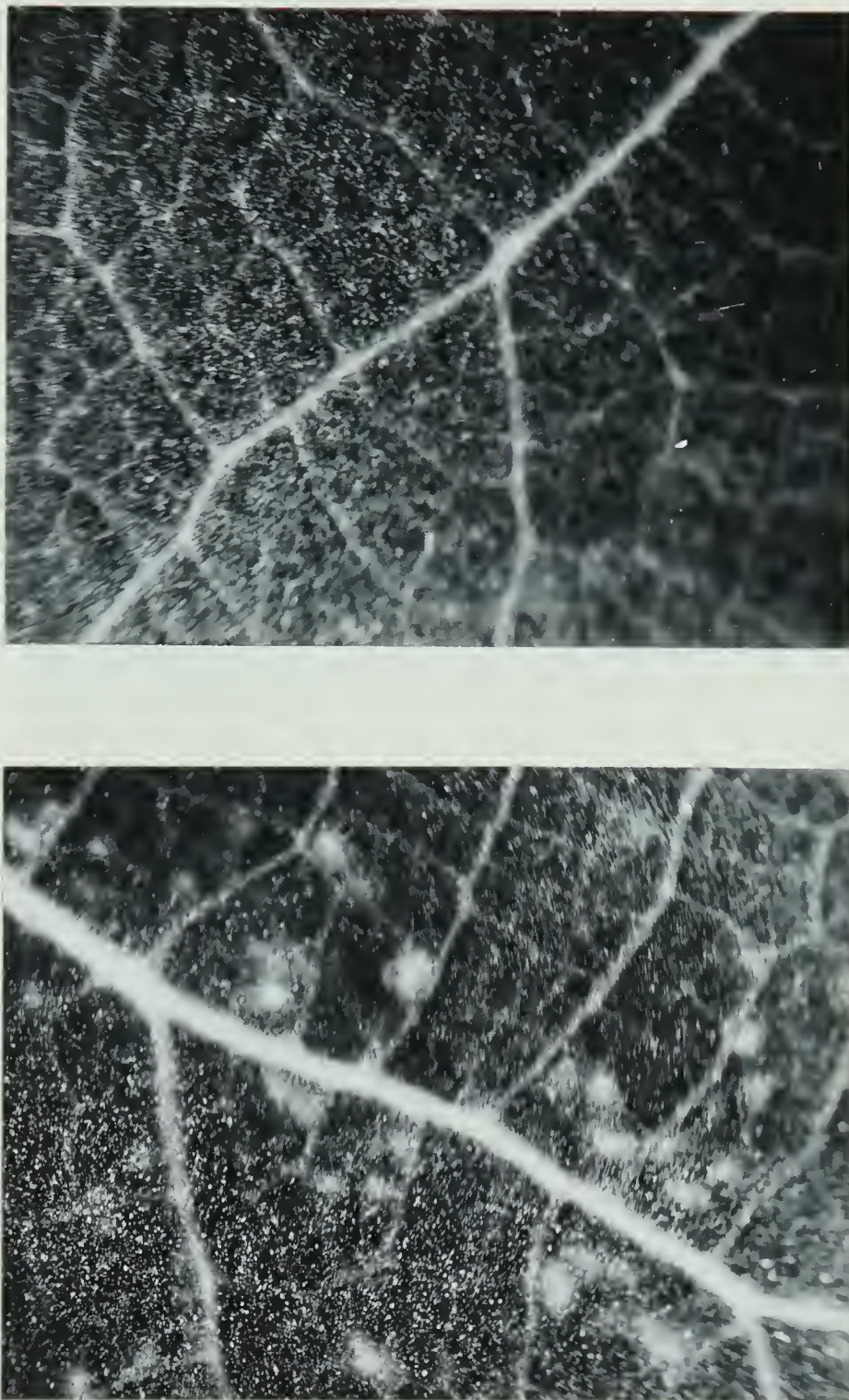


Fig. 8. Adaxial leaf surfaces of representative aspen poplar leaves in June (upper) and July (lower), photographed June 5 and July 5, respectively (magnification approximately 25X).

and where the number of these openings per unit area decreases as the leaf matures.

Influence of Temperature: Penetration of picloram and 2,4-D amine was examined at three temperatures: 10°, 25.5°, 40.5°C. Aspen poplar leaves from outside were collected in mid-July. Herbicides were applied without and with added surfactant under low and high humidity. Treatment periods ranged from 1 to 12 hours. The data in Table VI represent the percentage penetration of picloram and 2,4-D amine, with added surfactant, after 12 hours of treatment at the three temperatures.

Table VI: Penetration of picloram and 2,4-D amine with added surfactant, as a percentage of the dose applied (200 µg in 40 µl), 12 hours after application to the adaxial and abaxial leaf surfaces at three temperatures.

Relative humidity	Temperature	Picloram		2,4-D amine	
		Adaxial	Abaxial	Adaxial	Abaxial
Low	10°C	3.8	6.4	5.7	7.6
	25.5°C	9.5	9.9	11.7	13.9
	40.5°C	15.2	15.8	21.2	21.4
High	10°C	6.6	8.0	7.3	10.7
	25.5°C	15.0	20.6	25.6	26.5
	40.5°C	35.0	35.4	44.8	45.6

An increase in temperature resulted in sharply increased penetration of both picloram and 2,4-D under both low and high humidity. Penetration from adaxial surfaces increased three to five-fold between 10° and 45.5°C, two-to three-fold between 10° and 25.5°C. Increase in penetration between 25.5° and 40.5°C was slightly less than two-fold except for picloram under high humidity, where it was more than two-fold. Penetration from abaxial surfaces increased two-to four-fold between 10° and 40.5° and two-fold or less between 10° and 25.5°C. Penetration of both picloram and 2,4-D was

greater from abaxial surfaces than adaxial surfaces of the leaves at 10° and 25.5°C but at 40.5°C this difference was not observed. Thus, the influence of increasing temperature in enhancing penetration was more pronounced on adaxial surfaces than on abaxial surfaces of the leaf.

High humidity and temperature together were most effective in enhancing penetration of both herbicides (Table VI). Contact injury (consisting of chlorotic spots) at the treated sites on the adaxial and abaxial leaf surfaces was observed after 4 hours of treatment at 40.5°C (Fig. 9).

The influence of temperature on penetration from the adaxial surface of the leaves was also studied by using labeled picloram. Increase in temperature here also resulted in a marked increase in penetration (Table VII). Penetration of labeled picloram was lower than that of unlabeled picloram, both at 10° and 25.5°C (Table VI and VII). At 40.5°C, on the other hand, the rate of penetration of picloram* was greater than that of unlabeled material. No explanation can be offered for this greater penetration of picloram* at 40.5°C.

Table VII. Penetration of picloram* (0.1 μ C in 40 μ l) with added surfactant, as a percentage of the dose applied, from the adaxial surface of leaves, at three temperatures, under high humidity.

Time, hours	Temperature		
	10°C	25.5°C	40.5°C
2	--	1.7	6.1
4	0.6	1.7	12.8
8	1.2	2.5	26.6
12	1.7	3.7	37.5
24	2.4	6.6	--

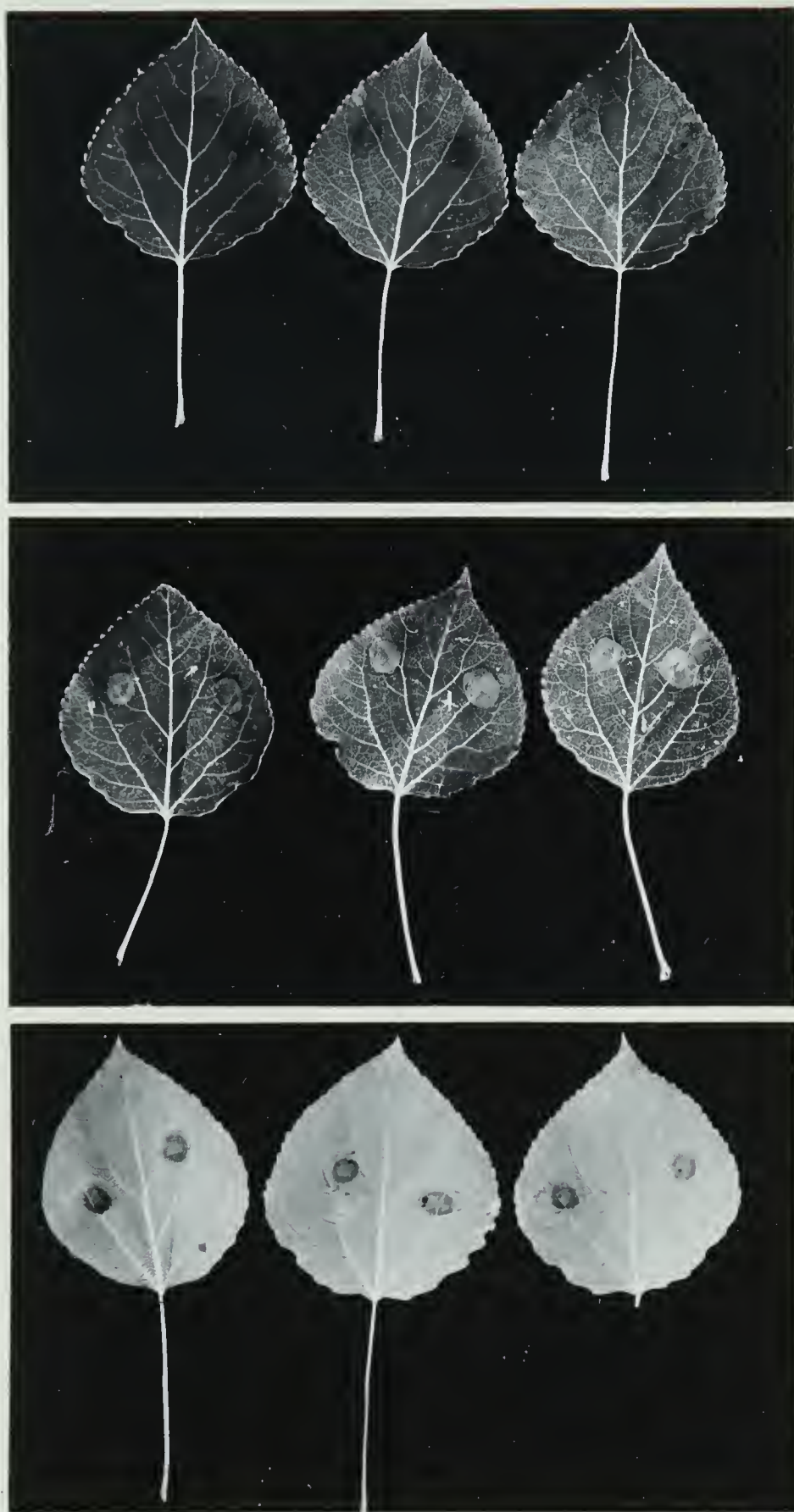


Fig. 9: Aspen poplar leaves showing contact injury at the treated spots on adaxial and abaxial surfaces after treatment with 2,4-D amine (1% surfactant added) at 40.5°C under high humidity.

Top: Adaxial surface, 4 hr.
Middle: Adaxial surface, 8 hr.
Bottom: Abaxial surface, 4 hr.

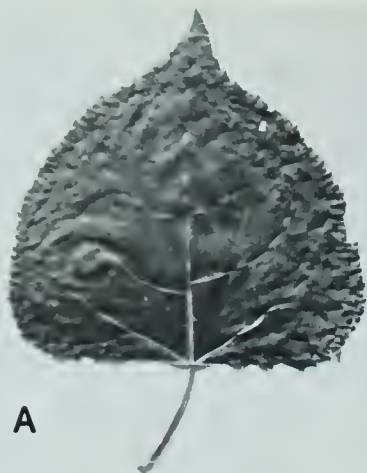
Higher temperatures may bring about a rapid movement of picloram* in the treated leaves, and thus help increase penetration. Autoradiographs of aspen poplar leaves treated with labeled picloram were prepared to examine this possibility. Picloram* with added surfactant (1%) was applied to the adaxial surface of the leaves at temperatures of 25.5° and 40.5°C, under high humidity. In the autoradiographs (Fig. 10) the dark image shows the location of the label whereas the faint images are due to excessive pressure in the leaf mount-film pack during exposure of the X-ray film (12). At 25.5°C very small quantities of picloram penetrated into the leaf, and movement in the leaf was relatively slow, in contrast to greater amounts of penetration and rapid movement throughout the leaf at 40.5°C (Fig. 10).

Influence of Pretreatment of Leaves with Chloroform: To examine the role of cuticular waxes in the permeability of adaxial leaf surfaces, some leaves were dipped in chloroform, a good organic solvent for cuticular waxes (17), for different periods of time. Such leaves were then treated with picloram and 2,4-D amine, without and with added surfactant, and penetration rates over a period of 8 hours were determined.

Prior dipping of the leaves in chloroform increased penetration of both picloram and 2,4-D by about one and a half-to four-fold, depending upon the time of dipping (Table VIII). An almost linear increase in penetration was obtained for dipping periods of up to one minute (Fig. 11). Further increases in dipping time resulted in a smaller increase in penetration. Added surfactant showed little increase in penetration of 2,4-D and a moderate increase for picloram. These results correspond with literature reports indicating that the removal of cuticular waxes from

Fig. 10. Mounts (left) and autoradiographs (right) of aspen poplar leaves treated with picloram* (0.1 μ c in 40 μ l), with added surfactant (1%), on the adaxial surface, at 25.5°C (A and B) and 40.5°C (C and D), under high humidity.

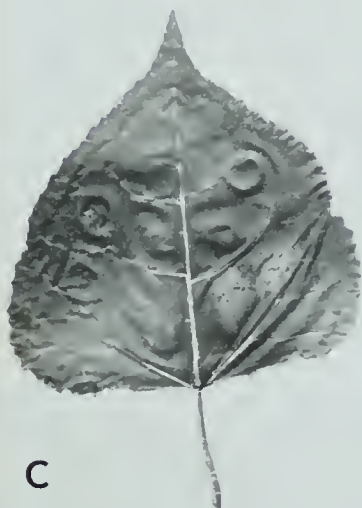
A: After 24 hours	B: After 48 hours
C: After 8 hours	D: After 12 hours



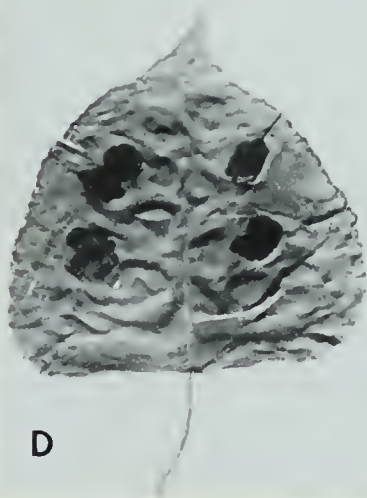
A



B



C



D



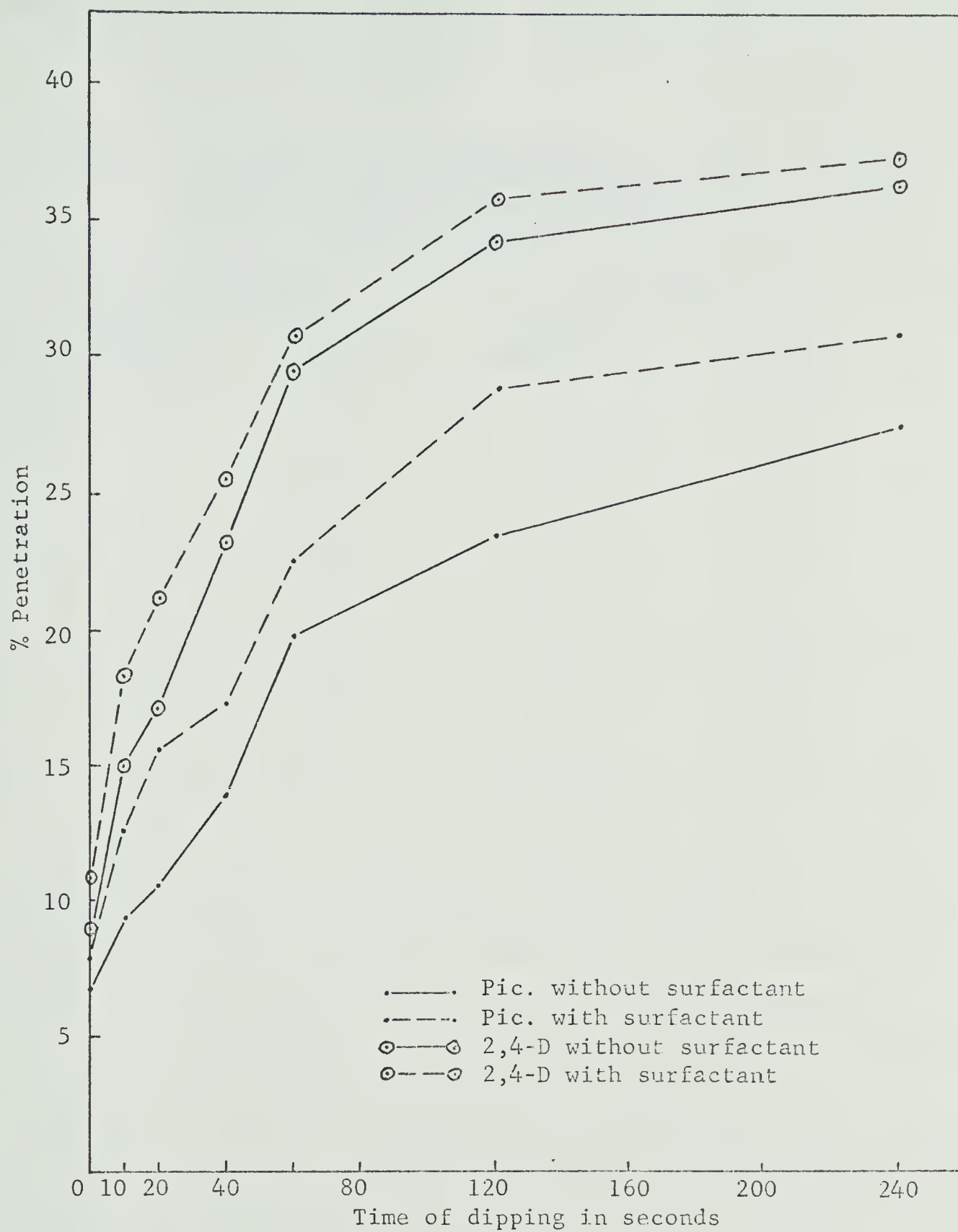


Fig. 11. Penetration of picloram and 2,4-D amine, with and without added surfactant, under high humidity, from the adaxial surface of aspen poplar leaves, following dipping in chloroform for varying periods.

isolated cuticle increases its permeability to plant growth regulators (10, 46).

Table VIII. Penetration of picloram and 2,4-D amine, as a percentage of the dose applied ($200\text{ }\mu\text{g}$ in $40\text{ }\mu\text{l}$), from the adaxial surface of chloroform-dipped leaves, after 8 hours of treatment under high humidity.

Time of dipping	Picloram		2,4-D amine	
	Without surfactant	With surfactant	Without surfactant	With surfactant
No dipping	6.8	8.6	8.8	10.6
10 sec.	9.1	12.5	15.1	18.6
20 sec.	10.5	15.5	17.0	21.1
40 sec.	13.8	17.2	23.2	25.6
1 min.	19.8	22.5	29.4	40.8
2 min.	23.5	28.8	34.2	35.8
4 min.	27.4	30.8	36.2	37.2

Influence of Concentration of Herbicide Solution: Aspen poplar leaves were treated with 40 or $100\text{ }\mu\text{l}$ of solution of picloram and 2,4-D amine, in 20 μl droplets. In each case the total dosage was $200\text{ }\mu\text{g}$ herbicide per leaf. The leaves were collected from outside around July 20.

An increase in volume of treatment solution resulted in only a slight increase in the amount of herbicide penetrated into the leaves (Table IX). Added surfactant and high humidity were equally effective in enhancing penetration at both the treatment volumes.

Table IX. Penetration of picloram and 2,4-D amine, without and with added surfactant (1%), as a percentage of the dose applied (200 μ g), from 40 or 100 μ l of solution (in 20 μ l drop-lets) after 12 hours of treatment under low and high humidity.

Relative humidity	Added surfactant	Picloram		2,4-D amine	
		40 μ l	100 μ l	40 μ l	100 μ l
Low	Without	4.0	4.6	10.0	9.3
	With	10.5	11.5	11.7	12.8
High	Without	9.9	10.7	20.4	21.8
	With	15.0	15.8	25.6	26.2

PENETRATION OF PICLORAM*IN BALSAM POPLAR:

Considerable difficulty was experienced in the spectrophotometric determination of residual herbicide from the leaves of balsam poplar. The presence of some 50% ethanol-soluble gummy material on the adaxial leaf surfaces resulted in very high and variable blanks. Therefore, experiments were conducted only with labeled picloram. Surfactant at 1% was included in the solutions. Treatments were applied at three different temperatures under high humidity.

An increase in temperature resulted in sharply increased penetration of picloram* in balsam poplar leaves (Table X). Penetration increased ten-to fifteen-fold between 10° and 40.5°C and two-to four-fold between 10° and 25.5°C, over a period of 2 to 24 hours after treatment.

Table X. Penetration of picloram* with added surfactant, as a percentage of the dose applied, from the adaxial surface of balsam poplar leaves, at different temperatures, under high humidity.

Time, hours	Temperature		
	10°C	25.5°C	40.5°C
2	--	--	9.8
4	1.2	2.2	18.3
8	1.6	4.8	33.0
12	2.2	7.3	40.0
24	3.1	10.9	--

The rate and amount of penetration of picloram* were greater in balsam poplar than in aspen poplar (Tables VII and X). Balsam poplar leaves are leathery in general appearance and may have thicker cuticle than aspen leaves. Thus one might expect less penetration of picloram* in balsam poplar leaves. Earlier studies (15) on comparative effects of picloram on these two species showed that balsam poplar is more resistant than aspen poplar to this herbicide. Our studies show that differences in penetration cannot account for differences in susceptibility to picloram between these species. Moreover, if the cuticle is thicker on balsam poplar leaves, then it also appears that not only the cuticle thickness, but also its composition and structure, as it varies between plant species, may play a significant role in penetration of leaf surfaces by herbicidal sprays.

PENETRATION IN CANADA THISTLE:

The influence of added surfactant, relative humidity, and temperature

on penetration of picloram and 2,4-D amine into leaves of intact Canada thistle plants grown in the greenhouse was examined.

Influence of Added Surfactant and Relative Humidity: Added surfactant at concentrations of 1% and 2% enhanced the penetration of picloram and 2,4-D amine (Fig. 12). An almost constant rate of penetration of 2,4-D amine with added surfactant was observed up to 12 hours after treatment. An increase of two-to three-fold due to added surfactant, for picloram, and two-fold for 2,4-D was obtained. Increase in concentration of surfactant from 1% to 2% resulted in little further increase in penetration of picloram and 2,4-D amine.

High humidity enhanced the penetration of picloram and 2,4-D amine (Fig. 12). Percentage increases were 40 and 22 for picloram and 2,4-D, respectively, with added surfactant at 1%. High humidity was much less effective in Canada thistle than in aspen poplar, where increases of 115 and 66 percent for picloram and 2,4-D amine, respectively, were obtained (Figs. 3 and 4).

Penetration of 2,4-D was greater than that of picloram, and it continued to occur at reasonably high rates up to 24 hours, while little penetration of picloram was observed after 12 hours (Fig. 12). Though the droplets of both herbicides dried in about the same length of time, the nature of the two chemicals may be an important factor in these differences in penetration.

In picloram-treated plants visible stem bending usually began 8 hours after application to the leaf. The degree of stem bending varied from 15 to more than 60 degrees, depending on the treatment. A similar response of Canada thistle plants to picloram was described by Chang (7). In spite

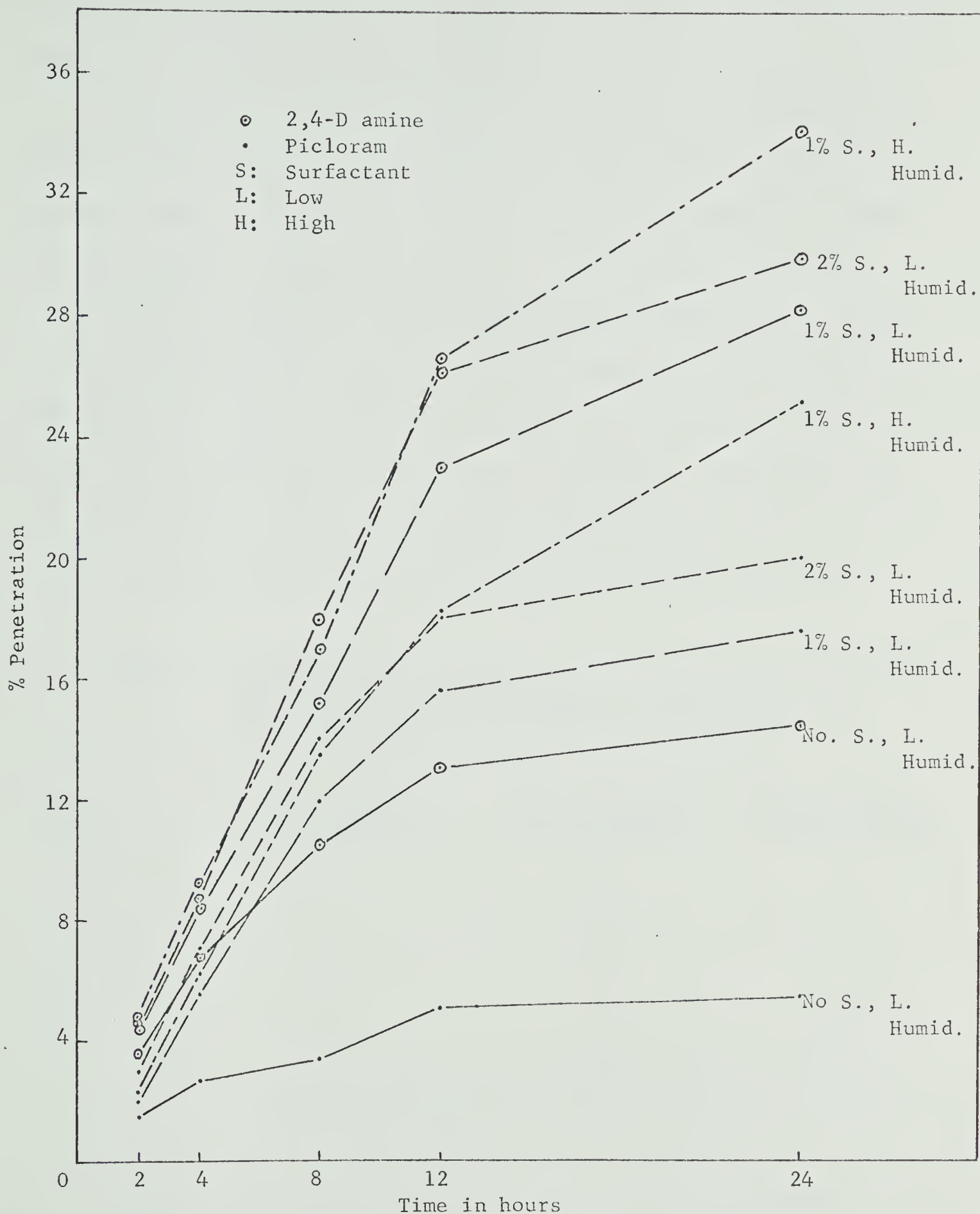


Fig. 12. Penetration of picloram and 2,4-D amine ($100\mu\text{g}$ dose/ $40\mu\text{l}$) with and without added surfactant Atlox 210 in Canada thistle, under low and high humidity.

of the low penetration rate of picloram, stem bending and leaf curling were more pronounced in picloram-treated than in 2,4-D treated plants.

The influence of added surfactant and humidity on penetration in Canada thistle plants was also studied by using labeled picloram. The results confirmed the earlier findings that added surfactant and high humidity enhance penetration (Table XI). In the presence of added surfactant the influence of high humidity became noticeable only after 12 hours of treatment. After 24 hours an increase of about 40 percent was obtained. Very small traces of radioactive picloram were also found in root extracts after 12 hours of treatment. No definite conclusions could be drawn from the latter observations, as even in later periods no appreciable amount of picloram* was detected in roots.

Table XI. Penetration of picloram*, as a percentage of the total dose applied ($.1 \mu\text{c}$ in $40 \mu\text{l}$) in Canada thistle as influenced by added surfactant and humidity.

Time, hours	Without surfactant, low humidity	With surfactant low humidity	With surfactant, high humidity
4	1.2	2.3	2.6
8	1.6	3.9	3.7
12	2.1	8.7	9.8
24	2.6	11.7	16.3
48	---	11.9	31.4

Because of the difference in locus of treatment application between aspen poplar and Canada thistle, a direct comparison of penetration in the two species cannot be made. However, penetration in Canada thistle was about twice as great as in aspen poplar from adaxial leaf surfaces. The

relative cuticle thickness in the two species may be an important factor, and may, in part, account for the observed differences in penetration. Moreover, in Canada thistle application of droplets on the mid vein might have resulted in greater penetration as the veinal regions are considered to be more easily penetrated by chemicals than are the inter-veinal ones (11, 65).

Influence of Temperature: Canada thistle plants were treated with picloram and 2,4-D amine solutions with added surfactant (1%), at three different temperatures: 10°, 25.5° and 40.5°C. An increase in temperature resulted in sharply increased penetration of both picloram and 2,4-D amine (Table XII). Penetration increased five-to six-fold between 10° and 40.5° C and nearly three-fold between 10° and 25.5° C. The relative increase was greater between 10° and 25.5° than between 25.5° and 40.5°C. At 40.5° C picloram-treated plants showed visible stem bending and leaf curling 4 hours after treatment, while no visible symptoms were observed at 10°C even after 12 hours. This observation suggests rapid entry of picloram into the leaves and also possibly rapid movement to other plant parts from the treated leaves at higher temperatures. In 2,4-D treated plants stem bending and leaf curling was noticed 12 hours after treatment at 40.5°C.

Table XII. Penetration of picloram and 2,4-D amine with added surfactant (1%), as a percentage of the total dose applied (200 µg in 40 µl), 12 hours after treatment at three temperatures under low humidity.

Temperature	Picloram	2,4-D amine
10°C	5.8	8.1
25.5°C	15.7	23.5
40.5°C	36.8	40.5

Experiments with labeled picloram on Canada thistle confirmed our findings on the influence of temperature on penetration of unlabeled material. Penetration of picloram* increased two-to fourteen-fold between 10° and 40.5°C and about four-fold between 25.5° and 40.5°C (Table XIII).

Table XIII. Penetration of picloram* with added surfactant (1%), as a percentage of the total dose applied (0.1 μ c in 40 μ l), at three temperatures under low humidity.

Time, hours	Temperature		
	10°C	25.5°C	40.5°C
2	--	--	4.8
4	1.2	2.3	9.3
8	1.8	3.9	23.3
12	2.2	9.8	36.2
24	3.0	11.7	--
48	--	11.9	--

DISCUSSION

The foregoing experiments have shed some light on the nature of foliar penetration, and on the influence of added surfactant and certain plant and environmental factors on such penetration of picloram and 2,4-D in aspen poplar, balsam poplar and Canada thistle.

Added surfactant enhanced the penetration of picloram and 2,4-D in all three species, though the magnitude of the increase varied. It is probable that the action of surfactant involves not only the intimacy of contact of the spray droplet with the leaf surface (13, 21, 62), but also (a), maintaining the herbicide in a fluid state as the aqueous carrier evaporates, as well as (b), the direct effect of the surfactant on the leaf surface and/or underlying tissue. In our investigations added surfactant provided a longer period for penetration of picloram and 2,4-D by maintaining the spray deposit in a fluid condition, and thus avoiding its crystallization. This is in accord with suggestions made by Jansen (40) and Sargent (55) that surfactants may act in an indirect capacity as humectants by retarding drying out of the solution. Hughes and Freed (39) were of the opinion that the energy required for absorption of a chemical from the fluid state is much less than that required for absorption from the crystalline state. Thus, hygroscopic surfactant would tend to hold water at the surface of the leaf, maintaining a fluid state which would tend to minimize the effect of humidity changes in penetration. Such an explanation is consonant with our findings of less increase in penetration by surfactant under high humidity than under low humidity.

A part of the beneficial action of extra surfactant (in addition to that provided by the surface-active agent already present in an

emulsifiable concentrate) could be its solvent action, since it is a good nonvolatile solvent for many substances. Temple and Hilton (64) pointed out that two solubility effects should be considered when a surfactant is included in a spray solution. One is the increased amount of herbicide dissolved in the initial spray solution and, as the water evaporates from the treated leaves, the other is a dynamic solubility equilibrium involving the surfactant, the remaining water, and the herbicide.

Added surfactant may influence directly the behavior of the cuticle. Jansen (40) postulated that one of the primary influences of the surfactant is promotion of hydration of the cuticle under adverse humidity conditions. Water from a surfactant droplet may be more readily available to the cuticle as a result of surfactant reduction of surface energy. However, if the surfactant also penetrates the cuticle, the hydrophilic-lipophilic nature of the bulky surfactant molecule dictates an even greater swelling and an increase in area of the hydrophilic channels and perhaps of the lipophilic portions as well. In addition, probably, surfactant swelling of the cuticle would be more lasting since the large surfactant molecules would tend to prevent complete contraction of the cuticle as water evaporates after treatment.

On the abaxial surface of the leaves added surfactant may further enhance the uptake of picloram and 2,4-D by promoting the stomatal component of penetration.

Our experiments indicated that relative humidity of the atmosphere that surrounds the leaves during the treatment period has a profound effect upon penetration of picloram and 2,4-D. The effect might be accounted for in several ways. For example, humidity may affect pene-

tration by influencing the rate of drying of the treatment droplets. It could very well influence the hydration of the cuticle and thus regulate the permeability of this barrier. It could modify the behavior of stomata and thus differentiate between a diffusional and a mass-flow of chemical into the leaf. A discussion of these possibilities follows:

High humidity during the uptake period probably increased penetration by increasing the time required for the droplets to dry. This possibility was confirmed in experiments under rewetted and high humidity conditions (Table 1). The results are in agreement with the findings of Pallas (48) and Prasad et al. (50) in similar experiments. Under high humidity cutin may absorb water and swell appreciably. This hydration, as suggested by van Overbeek (65), will spread the wax components farther apart, and this will have the effect of increasing the permeability of the cuticle. Thus, chemicals will better penetrate the water-saturated cuticle.

The cuticle, either external or internal, is very probably of a sponge-like consistency with the interstices filled with water when the plant and atmosphere are saturated, and filled with increasing amounts of air as the plant suffers water stress. Many investigators (48, 50, 70) have shown that surrounding the plant with a saturated atmosphere greatly facilitates penetration of, for example, 2,4-D, benzoic acid, dalapon and maleic hydrazide. Under high humidity conditions the spray droplet probably makes immediate contact with the continuous water phase, and the chemical is able to diffuse directly around and into the mesophyll cells via the cell walls. When the plant is under low humidity or water stress the menisci are pulled back into the minute capillaries in the cuticle and, as liquid is applied, air bubbles are entrapped in

the capillaries, blocking movement into the leaf.

On the abaxial surface of aspen poplar leaves, humidity may influence stomatal opening. Stomata are opened more widely under high humidity than under low humidity (3, 14, 48). If penetration occurs also through the stomata, such changes in stomatal openings may, in part, account for increased penetration of picloram and 2,4-D under high humidity. Under low humidity, with stomata mostly closed, the rate of penetration may be limited by the cuticle.

Finally, as considered by Prasad et al. (50), humidity might affect certain 'internal factors' related to water balance in the leaves, and hence modify the overall process of penetration.

Our results on the influence of growth stage which included, in penetration through the adaxial surfaces of aspen poplar leaves, an increase in July over that in June (Table V), do not correspond with the results of earlier investigations (26, 56, 57), in which penetration was found to decrease as the leaf matured and hence with increased cuticle thickness. However, mechanical damage of leaf surfaces as observed in our experiments, has been found to increase their permeability to herbicides (11, 38, 41) and can account for our results. Dalrymple and Basler (16) observed an increase in absorption of 2,4,5-T in black-jack oak in July over that in June, followed by a decrease in August and September. Although these authors did not offer an explanation of this seasonal variation, it is quite likely that physical changes in leaf surfaces as a result of seasonal variation resulted in the differences reported.

The observed slight decrease, with leaf age, in penetration from abaxial leaf surfaces (Table V) probably can be attributed to increasing

cuticle thickness as the leaf matures.

Penetration of picloram and 2,4-D was greater from abaxial than from adaxial surfaces of aspen poplar leaves. From adaxial surfaces (devoid of stomata) penetration may be primarily cuticular in contrast to both cuticular and stomatal penetration from abaxial surfaces of leaf. The stomata have been reported as ports of entry of chemicals having relatively low surface tensions (11, 13, 19, 65). If the open stomata are penetrated by herbicidal preparations (surfactants are included in commercial formulations), this could account for the greater penetration from abaxial than from adaxial leaf surfaces in our experiments.

Though the cuticle, in general, from both the leaf surfaces has been reported equally permeable to herbicides (11), the relative thickness of the cuticle and the presence of specialized parenchymatous tissue composed of thin walled cells near the abaxial surface may also be important factors responsible for differential penetration from the two leaf surfaces. The cuticle is generally considered to be thicker on the adaxial surface than on the abaxial surface.

Differences in penetration of the three herbicides included in this study are the results of differences in the nature of the herbicidal molecules. The polarity of herbicides determines their solubility in carrier solution and their compatibility with the cuticle and the cell wall. When penetration into and through fatty phases is involved, a non-polar compound has a better chance to penetrate than a relatively polar compound. For this reason, ester formulations of 2,4-D have shown more rapid and more extensive penetration in aspen poplar leaves than have other formulations. Because of its low polarity, 2,4-D ester

is compatible with the cuticle and can penetrate rapidly through the cuticle. Moreover, ester formulation of 2,4-D is both slightly water-soluble, and fat-soluble (65) and thus can follow both an aqueous and a lipoidal route through the cuticle. High volatility of 2,4-D ester may permit penetration of its vapors through the stomata.

Picloram (salt formulation) and 2,4-D amine, relatively polar molecules, are incompatible with cuticle, and primarily follow an aqueous route through the cuticle, thus resulting in lower penetration than that of 2,4-D ester. However, one may speak of the apolar properties of even polar compounds like 2,4-D amine and picloram, if these compounds are modified by the addition of surfactants which combine both polar and apolar properties. This would support our findings in which addition of surfactant resulted in a very great increase in penetration of picloram and 2,4-D amine compared with only a relatively small increase in 2,4-D ester penetration.

The effect of temperature on penetration might be explained if at least a part of the penetration process is visualized as an active process. On the basis of the relatively high temperature coefficients obtained, claims have been made that penetration is metabolically governed (31, 42, 55, 56). Undoubtedly, metabolic processes could exert an indirect influence on the penetration rate by influencing cytoplasmic viscosity, accumulation, binding and translocation of the penetrant. These processes will influence the concentration gradient across the surface layers. Temperature can also, however, directly influence the rate of diffusion of these chemicals through cuticle and other lipid-containing membranes. Van Overbeek (65) has pointed out that fatty molecules oriented in layers as micelles have a very low permeability at low

temperatures when the layer nears solidification, while at high temperatures these fatty substances become less viscous and offer less resistance to the passage of diffusates.

Prior dipping of the leaves in chloroform increased the permeability of leaf cuticle from adaxial surfaces to picloram and 2,4-D (Fig. 11). Short dipping periods, or treatment of cuticle disks in chloroform, have been reported to remove waxes, especially surface waxes, from the cuticle (17, 59). From our studies it appears that wax components of the leaf cuticle play a significant role in governing the permeability of aspen poplar leaves. Penetration of picloram and 2,4-D into the leaves was increased several-fold upon removal of surface waxes by short periods of dipping. Longer periods of dipping, which may extract the embedded waxes, resulted in only small further increases in penetration, suggesting that these embedded waxes are less important barriers to penetration than are the surface waxes.

The three plant species studied behaved differently in relation to herbicidal penetration. As stated earlier, a direct comparison of penetration in the three species is difficult to make because of the differences in locus of treatment application. However, at 25.5°C and under high humidity penetration of picloram* was greatest in Canada thistle followed by balsam poplar and then aspen poplar (Tables VII, X and XI). Variation in relative cuticle thickness in the three species may, in part, be responsible for the observed differences in penetration. Canada thistle leaves appear to have thinner cuticle than do aspen poplar leaves. One would expect a lower rate of penetration in balsam poplar than in aspen poplar, as balsam poplar leaves are leathery in appearance and possibly may have a thicker cuticle than aspen leaves.

But the results do not bear this out. It appears, therefore, that the composition and structure of the leaf cuticle may play a significant role in penetration of herbicides in aspen and balsam poplar leaves.

The enhancing effects of added surfactant, high humidity and temperature, were more pronounced in aspen and balsam poplar than in Canada thistle. All these factors primarily modify the behavior of leaf surfaces, especially the cuticle which is the first barrier to penetration of herbicide solutions. Accordingly, aspen and balsam poplar leaves, with relatively thick cuticle, show a greater response to added surfactant, relative humidity and temperature than do Canada thistle leaves.

Spectrophotometric and radioactive assay of picloram and 2,4-D were reasonably satisfactory methods in quantitative studies of penetration into leaves. Though the extent of penetration varied with the species studied, the location of plants from which leaves were collected, and the herbicide formulation, the results on relative response to various factors included in the spectrophotometric analyses were confirmed in experiments with radioactive herbicides. If the surface of the leaves has some material on it that appears in the washings (as in balsam poplar), spectrophotometric determination of residual herbicide from the leaves may not furnish satisfactory results.

CONCLUSION

All herbicides applied to leaves must traverse the cuticle and the epidermis, or move in through stomata to the chlorenchyma, in order to reach the phloem for long distance transport to roots and other plant parts. The three herbicides, picloram, 2,4-D amine and 2,4-D ester all possess the ability to enter leaves in this manner. Picloram penetrated most slowly, 2,4-D amine entered more readily, and 2,4-D ester penetrated most rapidly.

Added surfactant, Atlox 210, at a concentration of 1% enhanced the penetration of picloram and 2,4-D in aspen poplar leaves both under low and high humidity. It nearly doubled the penetration of picloram and 2,4-D amine while only a 20% increase was observed with 2,4-D ester. These findings confirmed that penetration was the chief factor responsible for the increased phytotoxicity that resulted from added surfactant in earlier experiments on aspen and balsam poplar (15). It appears, therefore, that herbicide doses may be reduced to a certain extent, without any appreciable loss in phytotoxicity, by the addition of this surfactant. A reduction in herbicide dosage would not only reduce costs, but also would minimize residue problems arising from applications of persistent herbicides such as picloram.

High relative humidity of the atmosphere that surrounded the leaves during the treatment period greatly enhanced the penetration of picloram and 2,4-D into leaves. The more water-soluble herbicides, picloram and 2,4-D amine, appear to vary more in effectiveness with variations in relative humidity than does the less water-soluble form, 2,4-D ester. Thus, high humidity during the spraying periods usually will be beneficial, for it prevents water stress in the plant, delays drying of spray droplets,

favours stomatal opening, and increases cuticular permeability.

Both the rate and amount of penetration of picloram and 2,4-D amine were greater from abaxial than from adaxial surfaces of aspen poplar leaves. The ester form of 2,4-D, a relatively nonpolar molecule, penetrated equally well from both leaf surfaces. The presence of stomata and the relatively thin cuticle on the abaxial surface are probably important factors contributing to this difference in penetration of picloram and 2,4-D amine from the two surfaces.

Penetration of picloram and 2,4-D in aspen poplar was fairly high at the beginning of the study in early June. There was an increase in penetration in early July, followed by a decrease in early August which continued till the end of September. Mechanical damage of the adaxial surface of the leaves in July was considered responsible for this unusual increase in penetration. Penetration from the abaxial surfaces decreased with advancement in growth stage. It is probable that increase in cuticle thickness with leaf age is responsible for this decrease in penetration. It appears, therefore, that herbicides should be applied early in the growth period, as soon as the leaves are fully expanded, to achieve maximum penetration. Spraying between mid-June and early July may be considered an optimum period for herbicide application for brush control.

Higher temperatures, 25.5°C (78°F) and 40.5°C (104°F), were distinctly superior to lower temperatures of 10°C (50°F). High temperature accompanied by high humidity resulted in the greatest amount of penetration. Under field conditions moderately warm, though not excessive, temperatures may be recommended for optimum spraying conditions. Herbicides should not be applied at temperatures of 50°F or

lower. Autoradiographs of the leaves showed not only that penetration was greater but also that picloram* moved much more rapidly and extensively in the leaf at 40.5°C than at 25.5°C..

Prior treatment of aspen poplar leaves in chloroform resulted in a several-fold increase in penetration of picloram and 2,4-D amine from adaxial leaf surfaces. The relative increase varied with the length of the dipping period. Short periods of dipping, up to one minute, resulted in a much greater relative increase than did additional periods, up to four minutes. The results with chloroform treatment show that inclusion of a wax solubilizer in herbicide formulation could increase the penetration rate and activity of nonpolar compounds.

Variation in concentration of herbicide solution at constant drop-let size and dosage per leaf did not result in marked differences in penetration of picloram and 2,4-D amine. Only a slight increase was observed with decrease in concentration. Under field conditions herbicidal effectiveness may be decreased as a result of runoff of the solution from leaf surfaces in high volume sprays. Moreover, a high spray volume would not be practical in aerial applications of herbicides for brush control.

In Canada thistle also, both added surfactant and high humidity enhanced penetration. An increase in surfactant concentration from 1 to 2% resulted in little further increase in penetration. High temperatures increased penetration of herbicides several-fold in both Canada thistle and balsam poplar.

Penetration of picloram from adaxial leaf surfaces was slightly greater in balsam poplar than in aspen poplar. It appears, therefore, that penetration does not play a significant role in the differences in

susceptibility to picloram between these two species. Studies on the effect of picloram on certain physiological and biochemical processes in aspen and balsam poplar plants and its metabolic behavior in these species may be of particular importance in correlating the differences in susceptibility between aspen and balsam poplar.

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APPENDIX

Table 1 Details of chemicals used

<u>Common Name</u>	<u>Trade Name</u>	<u>Chemical Name</u>	<u>Source</u>
Picloram	Tordon 22K	Potassium salt of 4-amino-3,5,6-trichloro-picolinic acid	Dow Chemical of Canada Ltd., Sarnia, Ontario
2,4-D amine	Weedar 80	Dimethylamine of 2,4-dichlorophenoxyacetic acid	Amchem Products, Inc., Ambler, Pa.
2,4-D ester	Weedone	Ethyl ester of 2,4-dichlorophenoxyacetic acid	Amchem Products, Inc.
	Atlox 210	A blended product containing polysorbate, mono-and di-glycerides, butylated hydroxyanisole, butylated hydroxytoluene and propyl glycol in a water-isopropanol medium.	Atlas Chemical Industries, Inc., Wilmington, Delaware.

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